JOURNAL OF DAIRY SCIENCE

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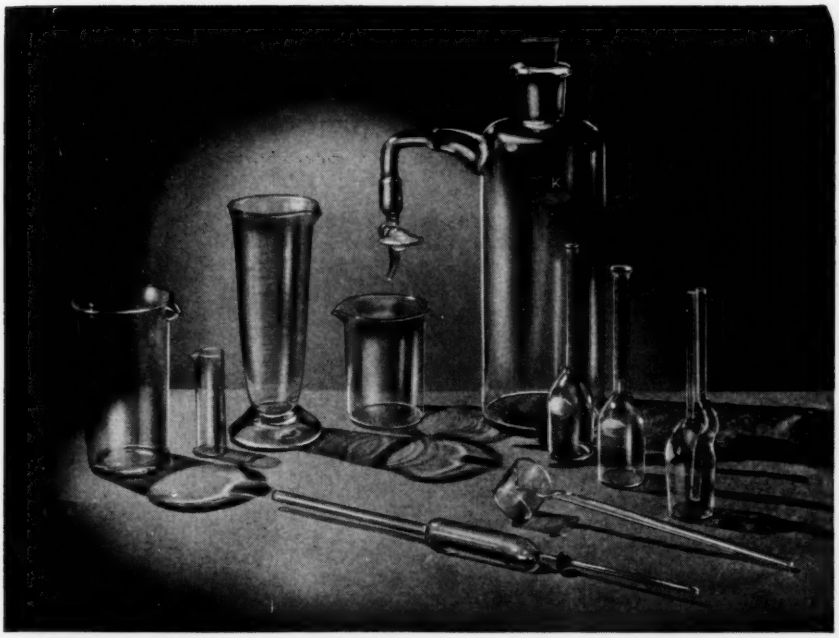

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JOURNAL OF DAIRY SCIENCE

VOLUME XXXIII JUNE, 1950 NUMBER 6

ABSTRACTS OF PAPERS PRESENTED AT THE FORTY-FIFTH ANNUAL MEETING

PRODUCTION SECTION

P1. A study of the inheritance of persistency in milk production. M. H. ALEXANDER, Univ. of Illinois

The data collected in this study show that, (a) the daughters of inbred sires vary less in their average persistency values (*P* values) than the daughters of non-inbred sires; (b) inbred daughters of each sire studied varied less in their *P* values than the non-inbred daughters of the same sire; (c) there is a distinct difference in the average *P* values of the cow groups by breeds; (d) there is a difference in the average *P* values between strains within the breeds; (e) there is a difference in the average *P* values of the daughter groups of different sires; (f) there is less variation in the *P* values of repeat pairs than of random pairs under the same environment; and (g) the regression of the *P* value of daughters on dams is consistent. From the results obtained in the analysis of these data it is concluded that persistency of lactation in dairy cows is a heritable factor.

P2. Some of the effects of calving interval on milk and butterfat production of Ayrshire cattle. W. J. TYLER AND GEORGE HYATT, JR., West Virginia University

The records used in this study were two-time M.E., 305 d. or less lactations taken from punch cards furnished by the Ayrshire Breeders' Association. There were a total of 2,203 animals studied. The average length of lactation was obtained by averaging the number of days in milk, with no cow being allowed more than 305 d. for a single lactation.

The data indicate that milk and butterfat production of cows with 10- or 11-mo. calving intervals is significantly lower than those having 12- or 13-mo. intervals. However, it is apparent that this lower production is due in part to a shorter average lactation. Study of the 2nd and 3rd lactations indicates that these cows with short calving intervals may be persistently poorer producers. The data also indicate that the unfavorable influence of a short calving interval may persist into the succeeding lactation. Significantly greater production was not obtained when cows had 14- or 15-mo. or longer calving intervals as compared with the 12- or 13-mo. intervals. The correlation between first and second calving intervals was found to be + 0.10.

P3. A study of the type ratings of daughters of sires and dams that have been classified for type. W. J. TYLER, West Virginia University

A previous study of official type ratings of Ayrshire cows indicated that the heritability of type was approximately 0.3. However, no estimate of the inheritance of the sire's type was made. Since some breeders of dairy cattle select for type on the basis of the type ratings of bulls and cows, it should be important to know the results of matings between sires and dams with the different classification ratings.

The first official and the highest official type rating of 2,005 daughters that were by 189 classified sires and out of classified dams have been studied to determine the probable effectiveness of selection for type in dairy cattle. The average rating of the daughters out of various combinations of classified sires and dams compared favorably with expected ratings computed from the heritability estimate of 0.3 and the difference between the parents and the average type ratings of the breed. These breed averages were 83.0 (first official rating) and 83.7 (highest official rating) for cows and 84.8 for bulls (based on Excellent = 92.5; Very Good = 87.5; Good Plus = 82.5; Good = 77.5; Fair = 72.5).

The results, when the highest official type rating was used, are summarized as follows: Excellent bulls mated to cows averaging 85.2 for type had daughters averaging 84.6; very good bulls mated to cows averaging 84.5 for type had daughters averaging 84.0; good plus bulls mated to cows averaging 85.0 for type had daughters averaging 83.4; good bulls mated to cows averaging 84.4 for type had daughters averaging 81.7. Use of the first official rating on each of the animals gave similar results.

P4 Preliminary report on the production records of crossbred dairy cattle. J. P. LAMASTER,

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G. W. Brandt and C. C. Brannon, S. C. Agr. Expt. Sta., and M. H. Fohrman, Bureau of Dairy Industry, U.S.D.A.

The cross-breeding of dairy cattle was started at this station in 1936. Two-breed females were produced by mating animals of different breeds. These two-breed females then were crossed with sires of a third breed to produce three-breed females. In a similar manner four- and five-breed females have been produced.

The crossbred group is handled in the same manner as the purebred groups in the station herd. They receive all the roughage they will consume and a concentrate mixture at the rate of 1 lb. of grain to 3 lb. of milk (4% F.C.M.). Production records are based on daily milk weights and monthly butterfat tests, for 305-day lactations. In December, 1941, all cows were changed from 3x to 2x milking. Production comparisons will be given for 13 two-breed crossbreds and their purebred dams; 7 two-breed females and their 13 purebred maternal sisters; 13 three-breed and 8 four-breed crossbreds and their dams. All sires, except one, have been proved in the station herd on purebred daughters. These proofs will be used in giving the results obtained to date.

P5. Inheritance of butterfat test in the Beltsville Holstein herd. Joseph B. Parker and Charles A. Matthews, U.S.D.A.

Butterfat tests were available on 361 Holstein cows in the Beltsville breeding herd. No selection has been practiced and all were tested for production on a standardized feeding and management basis. Correlation, analysis of variance and covariance have been used in an attempt to determine the inheritance of butterfat test. A highly significant correlation of 0.4850 was found with 316 dam-daughter pairs with a regression equation of $Y = 1.8564 + 0.5199X$. Analysis of variance shows a highly significant difference between the sires used. Allowing for the different butterfat test levels of the sires' mates, covariance shows highly significant differences between sires. Sorting by generations, where the use of sires overlap, gave highly significant differences by analysis of both variance and covariance. Cow-families show no significant differences. Postulating on heterozygous make-up with a minimum of 4 pairs of gene test factors, it is possible to approximate the actual results. Culling for low test would have increased the average test by only 0.04% and cut the numbers in half. On the basis of the present herd of 59 cows with completed records, there would only have been 33 cows testing 3.89%. The test of the 26 cows eliminated averaged 3.89%.

P6. A selection index for fat production utilizing the fat yields of the cow and her relatives. J. E. Legates and Jay L. Lush. Iowa State College

The object of this study was to devise an index for selecting dairy cattle more accurately by utilizing the fat yields of the individual and its relatives. Relatives considered were the cow's dam, daughters, maternal half sisters and paternal half sisters. Information about the fat yields of the cow and her relatives was combined in such a way that the index value for an animal was more highly correlated with its breeding value than if the information were combined in any other linear manner. Statistics needed to construct the index were calculated on an intra-herd basis; thus, the index is for selecting between individuals kept under the same general herd environment. These statistics were computed from an analysis of 23,330 lactation records from 12,405 cows in 293 Jersey herds on H.I.R. test during 1943 to 1947.

For heritability of observed differences in fat yield of 0.20, as determined from these data, information on the individual should receive about 2.75 times as much attention as the same information on the dam. Progress to be expected by using the index for selections between cows with records of their own would be about 1.10 to 1.15 times faster than by making selections on own performance alone.

P7. An index for the effects of certain environmental influences on dairy cattle production. N. D. Bayley and E. E. Heizer, Univ. of Wisconsin

This study is concerned with evaluation of the effects of specific environmental influences on sire provings. The data consist of information obtained on 967 cows in 47 different Holstein-Friesian herds of Wisconsin. The influences considered are condition before freshening as a heifer, age at first freshening, length of dry period, condition during dry period, age at last freshening, days with calf during lactation, length of lactation, lb. TDN daily/1000 lb. body weight, nutritive ratio of ration during barn feeding period of lactation and selection and number of milking cows in the herd. Ratings and scoring systems have been devised for influences not readily evaluated numerically.

Multiple regression techniques have been used to estimate the importance of the environmental factors and also the success of the devised measuring systems. The effects of the important factors have been combined in an index which expresses environmental variation in terms of butterfat. Further investigations concerning the use and validity of this index are discussed.

P8. Heritability of fertility in dairy cattle. R. S. DUNBAR, JR., AND C. R. HENDERSON, Cornell Univ.

Reduced fertility in dairy cattle is a serious economic problem, but little is known concerning the effectiveness of selection for high fertility and what relative emphasis in selection should be placed on fertility and on other important economic traits. Quantitative measures of the heritability of fertility and of the phenotypic and genetic correlations between fertility and other important traits are needed to answer these questions. Records of breeding performance and production of artificially sired cows offer an unusual opportunity to study these factors, as well as the joint effect of genetic "nicking" and genetic-environmental interaction.

Two measures of fertility, non-returns to 1st service and calving interval, have been employed in this study. By means of an estimation procedure which yields unbiased estimates of components of variance in non-orthogonal data, estimates were obtained of the variance due to additive genetic differences among sires. Sire variance was essentially zero in both studies. Therefore, heritability of fertility is estimated to be near zero.

From these results it is concluded that under the present conditions and using non-returns or calving interval as a measure of reproductive efficiency, selection for fertility in dairy cattle must be quite ineffective. Consequently, emphasis in selection should be placed on other traits of economic importance for which selection is known to be effective.

P9. Predicting the breeding efficiency of dairy cows. DURWARD OLDS AND D. M. SEATH, Kentucky Agr. Expt. Station

Data were tabulated from the herd record books of 20 local cooperative inseminating units of the Kentucky Artificial Breeding Association. Included in the study were 6,509 cows and 2,403 herds which were serviced both of 2 consecutive yr. It was found that 4,665 cows, each of which required only 1 service the 1st yr. averaged 1.44 services the 2nd yr.; 1,372 cows requiring 2 services the 1st yr. averaged 1.54 services the 2nd yr.; 400 cows requiring 3 services the 1st yr. averaged 1.64 services the 2nd yr. The 72 cows which required 4 services the 1st yr. averaged 1.65 services the 2nd yr.

The correlation between breeding efficiency for consecutive years was 0.084 ± 0.012. This correlation, though statistically highly significant, was too small to indicate a high degree of predictability.

The predictability of breeding efficiency of herds as units was about the same as that for cows. Only 9.3% of the "problem herds" (averaging 2.1 or more services/cow) were still problem herds the next year. The total number of problem herds was 7.1% the 1st yr. and 5.9% the 2nd yr. The average herd consisted of 7.1 cows the 1st yr. and 8.3 cows the 2nd yr.

P10. The frequency distribution of cellular antigens in five breeds of dairy cattle. L. C. FERGUSON, E. J. LAZEAR AND FORDYCE ELY, Ohio State Univ.

The recognition of more than 40 cellular antigens in the blood of cattle has contributed a tool for an analytical study of inherited characters. A study of the distribution of the antigens in the different breeds reveals additional evidence to support the idea proposed by Lush that breed differences are dependent on variations in the gene frequency in a given population. The paper of Owen, *et al.* (J. Animal Sci., 3:315. 1944) reported the observations on the frequency distribution of the cellular antigens in 2 breeds of dairy cattle. This report essentially confirms those observations and extends them to the other dairy breeds.

The results reported are based upon the test of 1,205 Holstein, 1,025 Guernsey, 443 Jersey, 211 Brown Swiss and 80 Ayrshire cattle with serum reagents for 36 to 40 different antigens. The data indicate some marked differences in the occurrence of certain antigens in the 5 breeds. Generally, the distribution of antigens is quite similar in the Jersey and Guernsey cattle tested. In the same way, there is a resemblance in the components of Holstein and Ayrshire cattle. There is a tendency for the frequency of the antigens in the Brown Swiss cattle to place this breed between the Guernsey and Jersey on the one side and the Holstein and Ayrshire on the other.

P11. Length of gestation in the five major breeds of dairy cattle. M. H. ALEXANDER, Univ. of Illinois

The Brown Swiss and Guernsey were shown to have a distinctly longer gestation period than the generally accepted 281-day average, while the Ayrshire, Holstein-Friesian and Jersey breeds were distinctly below this average. For all the breeds the gestation period for twins was less than for singles. Gestation periods preceding calvings in the summer months were somewhat shorter than for other seasons.

There is a slightly positive, but statistically insignificant, effect of increasing length of gestation on the following items which might affect production of milk and fat: (a) length of gestation

on persistency of secretion of milk and fat; (b) length of gestation on length of lactation; and (c) length of gestation on production of FCM.

The study shows conclusively that length of gestation is a heritable factor, since there is a distinct difference by breeds and since the grouped offspring of different sires within each breed show marked differences in time spent *in utero*.

P12. The preparation of a riboflavin-deficient milk for experimental calf feeding. E. G. Moody, S. M. Hauge and N. S. Lundquist, Purdue Univ.

Irradiation of skim milk with ultra-violet light yielded a product averaging 0.12 mg. riboflavin/l. (94% destruction) as determined by microbiological assay. Three gal. of milk in a glass jar, while being stirred, were treated with a 600 w. quartz mercury vapor lamp at a distance of 9 in. Five hr. irradiation at 60–65° C. produced the same destruction as 12 hr. at room temperature. Two infra-red and 2 flood lamps were used to supply heat but apparently had no photolyzing effect.

By means of chromatographic adsorption technique, using Florisil as the adsorbent, 98% of the riboflavin was removed from either skim or whole milk. This was accomplished in about 1/3rd the time required for irradiation and apparently did not change the flavor. Five gal. of milk were drawn with the aid of suction through a 3-in. column containing 1 lb. of Florisil. Whole milk required heating to about 45° C. The filtrate contained about 0.034 mg. riboflavin/l. as determined photofluorometrically.

P13. The influence of rumen inoculations on the digestibility of dry matter, cellulose and protein in young dairy calves. H. R. Conrad, J. W. Hibbs, W. D. Pounden and T. S. Sutton, Ohio Agr. Expt. Station

Thirty-two digestion trials were conducted to determine if calves inoculated with cud material from older cattle and fed rations high in roughages would be able to digest dry matter, cellulose and protein more efficiently than uninoculated calves.

Five inoculated and 5 uninoculated calves first were fed a ration of whole milk and alfalfa hay *ad libitum* for 14 d. During this period, the calves which were inoculated digested a significantly higher percentage of ingested cellulose (difference 4.81%) and dry matter (difference 3.84%) than the calves which were uninoculated. However, this appreciable difference in digestibility between inoculated and uninoculated calves disappeared when some of these calves later were placed on rations of either alfalfa hay or grass clippings only. No differences were found in the apparent digestibility of protein on any of the rations.

A calf which previously had been freed of characteristic "indicator" rumen microorganisms by heavy grain feeding showed a marked decreased ability (11.26%) to digest cellulose when placed on an alfalfa hay ration, in contrast to a similarly treated calf in which the characteristic microorganisms had reappeared, possibly through natural reinoculation.

P14. Variations in digestibility of certain characteristic rumen microorganisms and some effects of their absence on calves. W. D. Pounden and J. W. Hibbs, Ohio Agr. Expt. Station

Contents of various parts of the digestive tract of cattle were examined for the presence of usual varieties of rumen microfauna and 4 species of microflora which characteristically were present in their rumens. The 4 microflora types were larger coccoids, large cigar-shaped rods, small rods in flat rectangular groups and large thick square-ended rods.

The disappearance of usual rumen protozoa and the large cigar-shaped rods from abomasal and intestinal contents, as reported by others, was confirmed in this study. Some of the larger coccoids were observed in all parts of the digestive tract. The small rods and the large thick rods appeared gradually to disintegrate as they reached posterior parts of the tract. The ultimate fate of rumen microorganisms thus appears to vary between the extremes of complete destruction in the abomasum to passage entirely through the tract.

A Jersey calf was raised to 6 mo. of age with a rumen free since birth of usual rumen protozoa but containing massive numbers of the large cigar-shaped digestible rod. It had a neat and healthy appearance like 12 similar calves which received rumen inoculations and which were fed similar feeds. Three other calves whose rumens were devoid of both usual protozoa and the 3 rods but which contained the larger coccoids had rough hair coats and their abdomens appeared abnormally deep and pot-bellied.

P15. Some chemical and physical characteristics of the contents of the alimentary tract of calves at time of birth. D. B. Parrish and F. C. Fountaine, Kansas Agr. Expt. Station

At time of birth of the calf all sections of the alimentary tract contain a mixture of fluids and various solids, including hair. Total weight of alimentary contents obtained from 5 calves ranged from 609–958 g.; dry matter from three calves, 113–168 g. Average pH of the contents decreased in the following order: stomach, cae-

cum, small intestine, large intestine and colon. In general, the nearer the anus the contents were found, the greater the percentage of dry matter and the darker the color. Dry matter of stomach contents was 1.4% and of colon contents 26.5%. Average percentage of various substances found in the meconium from the large intestine was crude protein, 11.5; ether extract, 2.9; ash, 1.1; and N.F.E., 10.1. Ions identified in the ash of the meconium were chlorides, sulfate, carbonate, phosphate, silica, sodium, potassium, calcium and magnesium. Results of other analyses will be presented.

P16. Postpartum development of bovine stomach compartments and observations on some characteristics of their contents. SIDNEY P. MARSHALL, P. T. DIX ARNOLD AND R. B. BECKER, Univ. of Florida.

Jersey male calves have been anesthetized, the thoracic and abdominal cavities opened, apertures to the rumen, reticulum, omasum and abomasum closed by clamps and the stomach removed. Volume displacement of each compartment and of tissue of each compartment were measured, tissue and contents of each compartment weighed and pH and specific gravity determined where adequate quantities of materials were available.

Weight and volume of rumen tissue and weight of fresh contents exceeded that of the abomasum between 7 and 30 d. of age. Omasum and reticulum development also proceeds rapidly in young calves. Aliquots of stomach compartment contents taken in open containers for pH analysis ranged as follows: rumen, 5.17–6.89; reticulum, 5.19–7.19; omasum, 4.98–6.81; and abomasum, 2.1–4.61.

Consumption of grass, leaves, hay, excelsior bedding and sand occurred in increasing amounts during early life, as indicated by their presence in the stomach compartments.

P17. The growth of dairy calves on purified diets. G. P. LOFGREEN, Univ. of California

The response of male dairy calves to two purified diets has been determined. The diets differed in their mineral composition and the methods used to dissolve the casein and to emulsify the fat.

In one diet the casein was dissolved in $NaHCO_3$, the fat was homogenized into the mixture and the entire mineral mixture added at one time. Milk prepared in this manner always contained a heavy precipitate of minerals. Calves fed this diet grew at a subnormal rate, scoured more frequently than the control calves and frequently showed loss of hair.

The second diet was made by dissolving the casein in $Ca(OH)_2$, adding the minerals in steps from various mineral solutions and emulsifying the fat by use of lecithin or commercial non-ionic emulsifying agents. This milk had no precipitate, thus facilitating the feeding on an homogenous mixture. Calves fed this diet scoured less, showed less loss of hair, but gained at a subnormal rate.

In both groups the critical period appeared to be the first 3 wk. During this period, calves either lost weight or merely maintained their weight. From 3–8 wk. the gains approached normal.

P18. The nutritive value of starch and the effect of lactose on the nutritive values of starch and corn syrup in synthetic milks for young calves. R. J. FLIPSE, C. F. HUFFMAN, C. W. DUNCAN AND H. D. WEBSTER, Michigan Agr. Expt. Station

Nine neonatal calves, 6 Holstein, 2 Jersey and 1 Ayrshire, were allotted to 3 experimental groups and fed rations consisting of synthetic milks which varied only in the carbohydrate component. Calves were separated from their dams at 4–12 hr. after birth, placed in individual pens and started on the synthetic milk diet after fasting for 24 hr.

Calves receiving corn syrup plus lactose (KL) gained an average of 28.33 lb., those receiving starch plus lactose (SL) gained an average of 24.67 lb., while calves on starch (S) averaged only a 14.00-lb. gain in 31 d. KL and SL calves gained uniformly throughout the trial; S calves gained little or none the first 2 wk. but gained rapidly the latter half of the trial. The efficiency of feed utilization, expressed as pounds of gain per pound of dry matter consumed, was 0.487, 0.412 and 0.204 for the KL, SL and S groups, respectively. Diarrhea was much more common in the S group than in the other two groups.

Serial blood samples were collected before a test meal and at 0.25, 0.5, 1, 2, 4, 6 and 8 hr. after feeding and analyzed for blood sugar. The blood sugar level rose rapidly after the ingestion of glucose, lactose or corn syrup, with the maximum concentration at 4, 4 and 1 hr. after feeding, respectively. Following starch ingestion, there was no change in blood sugar the first 4 hr. and only a moderate increase at 6 and 8 hr.

P19. Is A.P.F. of value in a calf starter for calves weaned from milk at an early age? L. L. RUSOFF AND M. O. HAQ, Louisiana State Univ.

A 19.4% digestible protein calf starter containing additional thiamine, riboflavin, calcium pantothenate and niacin, but no animal protein,

was compared with a similar ration supplemented with A.P.F.[1] so that the ration contained 10 mg. of vitamin B_{12} per ton. The A.P.F. did not appear to be of any value for calves weaned from whole milk at 28 d. of age, when gains in weight, height at withers and feed efficiency were compared at 63 d. of age. The decrease in growth rate which always occurs after weaning calves from milk at an early age was not prevented by the feeding of A.P.F.

This experiment is still in progress. Results at the completion of the experiment (90 d. of age) will be available at the time of the meeting.

[1] A.P.F. no. 3. Courtesy Merck & Co., Rahway, N. J.

P20. APF supplements in milk replacements for dairy calves.[1] J. B. Williams and C. B. Knodt, Pennsylvania Agr. Expt. Station

Fifteen male Holstein calves, 7 d. of age, were divided into 3 comparable groups on the basis of height at the withers, body weight and chest circumference. All calves were fed the basal replacement mixture composed of the following ingredients: dried skimmilk, 50 lb.; dried whey, 10 lb.; dried distiller's corn solubles, 10 lb.; soluble blood flour, 10 lb.; ground oat groats, 5 lb.; cerelose, 7.75 lb.; dried brewer's yeast, 4.9 lb.; irradiated yeast (9F), 0.10 lb.; stabilized vitamin A feed (2,220,000 U.S.P. units/lb.), 0.22 lb.; mineral mix, 0.042 lb.; and dicalcium phosphate, 2.5 lb. Group I was fed the basal replacement, group II—basal replacement plus 2.2% APF supplement (3–4 μg./g. B_{12} equivalent) and group III—basal diet plus 0.3 per cent APF supplement (27.5 μg./g. B_{12} equivalent). The calves also were fed a suitable calf starter and good quality mixed hay *ad libitum*. The rate of feeding of the replacement was the same for all calves. Under the conditions of this experiment, it does not appear that the supplementation of the milk replacement used with APF improved the rates of growth in Holstein bull calves.

[1] The cooperation of Merck & Co., Rahway, N. J. and Lederle Laboratories, Pearl River, N. Y. is acknowledged and appreciated in the conduction of this trial.

P21. Serum protein fractions of calf blood as influenced by colostrum and skim milk. R. F. Elliott and Cecil Conley, Kentucky Agr. Expt. Station

Whole colostrum or colostrum cream in skim milk was fed to each of 9 calves for 2 d. The calves were bled periodically and the serum protein fractions estimated colorimetrically after the method of Wolfson *et al.* (Am. J. Clin. Path., 18: 723. 1948). At birth, the average values found for total protein were 4.0 g./100 ml. serum and 2.37, 1.66, 0.68, 0.82 and 0.20 for albumin, total, alpha, beta and gamma globulins, respectively. Following the consumption of colostrum, immediate increases of the various fractions were observed with maximum values being reached within 20–30 hr. after birth. The various fractions then leveled off with some fluctuations and with no tendency to decrease. Calves which were fed the skim milk did not show immediate increases in serum protein fractions and only a slight tendency to increase to 10 d. of age. The gamma globulin fractions for these calves were extremely low. As compared to normal calves, the average total serum protein of new born calves was two-thirds as great, while the average globulin fractions were only half as great as those observed for calves 2 mo. of age. Calves fed milk from their prepartum-milked dams follow the same pattern in respect to serum protein fractions as those observed for the skim milk-fed calves.

P22. The effect of kind of carrier and method of dispersion on the absorption of carotene by young dairy calves. J. W. Crowley and N. N. Allen, Univ. of Wisconsin

Dairy calves, fed from birth on a carotene-free ration of goat colostrum and goat milk, were used to study the absorption of carotene. Their blood carotene was very low, and by partially substituting skimmed goats milk, the plasma vitamin A was kept at about 10 μg./100 ml.

For each treatment, a single feeding of 100 mg. of crystalline carotene was dispersed in 35 ml. of the oil being tested, which was fed to the calves in skimmed goats milk. Two methods of dispersion and 5 different oils were compared. Blood samples were collected before treatment, every 4 hr. after treatment for 24 hr. and every 24 hr. thereafter until the blood carotene had decreased to near the pre-treatment level.

Five carriers (corn, soybean, cow butter, goat butter and mineral oils) were used. When dispersion was accomplished by stirring, the average maximum carotene increases in 5 Guernsey calves for the 5 oils were 25, 26, 24, 28 and 8 μg./100 ml. of blood plasma, and for 5 Jerseys, 24, 24, 25, 32, and 7, respectively; when homogenization was used, Guernseys increased 65, 69, 69, 75 and 13, and the Jerseys increased 72, 69, 70, 80, and 14, respectively. The maximum increase was reached in 12–16 hr. and required 2–3 wk. to return to the pre-treatment level. There was no change in blood vitamin A follow-

ing treatment. Due to difficulties in making quantitative collections of feces, excretion data are not considered reliable, but in general, about two-thirds of the carotene fed was recovered in the feces for the 1st 4 oils and a greater amount for the mineral oil.

Much of the carotene appears to be absorbed as such and to linger in the blood of calves without being converted to vitamin A, even though the calves are at a low plane of vitamin A nutrition.

P23. The riboflavin requirement of the very young calf. GERMAIN J. BRISSON AND T. S. SUTTON, Ohio State Univ.

Nine dairy calves were used to determine the minimum riboflavin requirement for the calf up to 8 wk. of age. The calves were taken from their dams at birth and fed colostrum for 2 d., after which they were fed a photolyzed whole milk ration supplemented with vitamin A and with riboflavin at different levels. The riboflavin was supplemented at the levels of 0, 25, 35, 45 and 115 μg./kg. of body weight daily. The latter calf received the same amount of riboflavin as a similar calf fed an untreated whole milk diet. At the 0 and 25 μg. levels, growth was reduced and deficiency symptoms appeared. At the 35 μg. level, growth was apparently normal and practically all deficiency symptoms were eliminated. At the 45 μg. level, performance of the calves was apparently normal. The average daily urinary excretion of riboflavin per 100 lb. of body weight was 0.06, 0.11, 0.12, 0.40, 2.66 and 2.29 mg. for the calves receiving 0, 25, 35, 45 and 115 μg. of riboflavin/kg. and untreated milk, respectively. On the basis of these results, it is concluded that the minimum daily riboflavin requirement for the very young dairy calf is between 35 and 45 μg./kg. of body weight.

P24. Synthesis of certain B-vitamins in the digestive tract of dairy calves. E. M. KESLER AND C. B. KNODT, Pennsylvania State College

Levels of thiamine, riboflavin and nicotinic acid were determined on the contents of the rumen, omasum, abomasum, small intestine and large intestine of male Holstein calves. In the 1st part of the experiment, 2 16-wk.-old calves were slaughtered at 2, 4, 6, 8 and 12 hr. after feeding, respectively. Concentrations of the 3 B-vitamins were found to be higher in all parts of the digestive tract than in the feed ingested on a dry matter basis. There was a decided peak in thiamine and nicotinic acid concentrations at 6 hr. after slaughter. Levels of riboflavin and nicotinic acid were much higher in samples from the small intestine than in other parts of the digestive tract.

In the second trial, 3 calves were slaughtered in each of the following age groups: 2, 4, 6, 8, 10, 12 and 14 wk. These calves were fed a maximum of 300 lb. of whole milk and calf starter up to 5 lb./d. with alfalfa hay *ad lib.* B-vitamin levels generally were higher on a dry matter basis in all parts of the digestive tract than in the feed given. This was true of all ages studied. Similarly, levels of riboflavin and nicotinic acid were higher in the small intestine than in the other regions studied. Much individual variation was noted throughout.

P25. The effects of estrogen and progesterone on the arterial system of the uterus of the cow. WILLIAM HANSEL, Cornell Univ.

The arterial systems of the uteri of 7 normal cows and 7 ovariectomized cows treated with varying amounts of estrogens and progesterone were studied histologically and by means of plastic injection-corrosion preparations. The arterial systems of the uteri of 16-mo.-old heifers and mature cows were found to differ markedly. The endometrial arterioles in the uteri of heifers were found to be essentially straight, while similar arterioles in the uteri of mature cows were found to be coiled on their central ends. Tightly coiled endometrial arterioles were found in the caruncules.

The degree of development of the arterial systems of the uteri of the ovariectomized cows was correlated with the amounts of the ovarian hormones administered during the 17-mo. period prior to the time they were slaughtered.

P26. The fertility of heifers following administration of progesterone to alter the estrual cycle. E. L. WILLETT, American Foundation for the Study of Genetics, Madison, Wis.

A study has been made to determine the fertility of yearling heifers inseminated artificially at the 1st heat following injection of progesterone to alter their estrual cycles. Fifty to 100 mg. of progesterone were injected daily starting on the 14th or 15th day of the cycle and continuing for 13–17 d. During estrus, each animal was bred when heat was first noted and again 24 hr. later. Fourteen heifers received 1 treatment and 4 received a 2nd when they failed to conceive the 1st time.

Estrus was observed, on the average, 5 d. following termination of injection (range: 4–7 d.). The resulting cycles varied from 28–34 d. in length. Eleven pregnancies were obtained from the 22 breedings. The inseminations for the 1st 13 breedings were performed by an inexperienced inseminator, and 5 or 39% resulted in pregnan-

cies. The last 9 were performed by an experienced inseminator, and 6 or 67% resulted in pregnancies. These results are within the range of breeding efficiency for heifers of this age as reported in the literature. The work is being continued.

P27. A preliminary report on the role of progesterone in the maintenance of pregnancy in the cow.[1] JAMES I. RAESIDE AND C. W. TURNER, Missouri Agr. Expt. Station

An attempt has been initiated to determine, quantitatively, the progesterone required for the maintenance of pregnancy in dairy heifers. Removal of the corpus luteum from 3 Holstein heifers in the early stages of pregnancy was performed by manual expression through a flank incision made after a positive diagnosis by rectal palpation. Daily subcutaneous injections of 25 mg. progesterone in olive oil were commenced 1 d. prior to the operation.

While the estimated ages of the foetuses at the time of the operation were 44, 48 and 76 d., abortion occurred in all 3 instances. By close observation it was possible to recover the foetuses at abortions 6, 16 and 12 d. after the respective corpus luteums had been removed. From this, it was concluded that 25 mg. progesterone daily was insufficient to maintain the pregnant condition at this stage. Increased amounts of progesterone will be tested until the pregnant condition can be maintained.

[1] Contribution from the Department of Dairy Husbandry, Missouri Agr. Expt. Station, Journal Series no. 1206.

P28. Relative reactions of European and Indian cattle to changes in environmental temperature.[1] S. BRODY, H. H. KIBLER AND A. C. RAGSDALE, Missouri Agr. Expt. Station, and H. J. THOMPSON, U. S. D. A.

The zone of thermoneutrality for lactating cows under normal conditions is between 40 and 60° F. The increase in heat stress with increasing temperature up to 105° F. is much greater than the increase in cold stress with temperature decreasing to 0° F. and is much greater in European than Indian cows. Appreciable change with rising temperature occurred at the following temperatures: rectal temperature, 75° F. in European and 95° F. in Indian; pulse rate, 85° F. in European and 95° F. in Indian; respiration rate, 65° F. in European and 80° F. in Indian; hay consumption, 75° F. in European and 85° F. in Indian; the rate of change of these reactions was always steeper in European than Indian cows. Unlike in (sweating) man, surface temperatures in European cows exceeds 95° F. when the environmental temperature exceeds 99° F. and the two meet at about 105° F. The water consumption above 85° F. increased in the Indian and decreased in European except Jersey 212. Gradually decreasing temperature from 50 to 5° F. did not affect the rectal temperature, but increased the heat production in Jersey and Holstein cows from 20 to 30%, more in small than large cows.

[1] Contribution from the Missouri Agricultural Experiment Station and the U. S. Dept. of Agr. (Mo. Sta., Journal Series 1203).

P29. A study of the effect of two- and three-times-a-day milking upon milk yield. J. G. CASH AND W. W. YAPP, University of Illinois

The chief objective of this experiment was to make a comparison between 2- and 3-times-a-day milking when environmental factors remained constant. After balancing the 2 sides of the udders as to yield, one-half of the udders were milked twice daily and the other 3 times. The experiment was conducted throughout complete lactation periods. Eight lactations on 7 different cows, consisting of 5 Holsteins, 1 Guernsey and 1 Ayrshire were studied. All cows used in the experiment were fed 3 times daily.

The total production of milk from the sides of the udders milked 3 times daily was 132% of the production from the sides milked twice daily. As lactation progressed, the percentage difference between the production of the halves of the udder milked 2 and 3 times daily became greater.

The average per cent fat content for the lactations was 3.68 for the milk from the sides of the udders milked 3 times daily and 3.63 for the milk from the sides milked 2 times daily.

P30. Observations on the rate of milk removal. K. E. HARSHBARGER, University of Illinois

The rate of milk removal during the milking process for individual cows milked in a milking parlor under standard conditions has been determined. The time between application of teat cups and initial flow and the cumulative amount of milk removed at intervals of 20 sec. were recorded. The rate of removal was calculated from an adjusted "end-point" of milking. The observations were taken on 2 d. of each month for the cows studied throughout a lactation period. Differences in the rate of removal between cows at various levels of production and within cows throughout the lactation have been evaluated.

The rate of milk removal increased with higher milk production and the total time re-

quired to milk high-producing cows was not proportionally higher than that of low-producing cows. The rate of removal for individual cows decreased throughout the lactation period along the same trend as the decrease in milk production.

P31. The utilization of acetic acid by the perfused mammary gland. G. L. McClymont and J. C. Shaw, Univ. of Maryland

The mammary glands of 4 cows were perfused with blood to which acetic acid was added. An artificial heart-lung was used to perfuse the glands. Acetic acid was determined on the half of the udder not perfused for a control and on the perfused half at the end of the perfusion. The blood was analyzed for acetic acid at the beginning and end of the perfusion. The utilization of the 4 glands in terms of 100 ml. of blood/100 g. of tissue was 0.17, 0.09, 0.10 and 0.16 mg., respectively.

P32. Variations in residual milk obtainable by oxytocin injections. Eric W. Swanson and S. A. Hinton, Univ.of Tennessee

Residual milk was secured following a normal milking by injecting Pitocin intravenously and resuming milking. Ten cows were sampled at monthly intervals and 5 others were sampled only once at various stages of the lactation. The quantity of residual milk obtained bore a close relationship to the normal milking ($r = 0.43$). It was highest at the 2nd mo. (the peak) of lactation and declined from the peak average of 5.7 to 2.5 lb. in the 10th mo. The change in residual milk, with progressive lactation, may be due to a decreased active gland size, more complete excretion or a combination of these factors. The butterfat percentage in the residual milk was highest in early lactation and declined proportionally with quantity as lactation advanced. The highest monthly average was 16.4% and the lowest 11.6%. The fat content of residual milk from Jerseys and Holsteins bore the same relationship as the fat tests of their normal milks. The solids-not-fat percentage of the residual milk changed only slightly throughout the lactation. While the fat content of the milking following oxytocin-stripping was depressed 1.3%, the solids-not-fat content was not changed significantly.

P33. Evaluation of mammary gland development of heifer calves. J. H. Book, W. W. Swett and C. A. Matthews, U.S.D.A.

The technique of examination and evaluation of mammary gland development of dairy calves has now been applied to over 300 Holsteins and Jerseys in the Bureau of Dairy Industry Herd at Beltsville, Md., which have completed production records. For most of the ages studied (3, 4, 5 and 6 mo.), the grades assigned to the calves and their highest mature equivalent milk production records showed highly significant correlations. The grade assigned at 4 mo. of age gave the highest correlation for Jerseys and the grade at 6 mo. of age gave the highest correlation for Holsteins. There are some indications, however, that averaging the grades for 2 or more consecutive mo. increases the reliability of grades for mammary development as an indication of production. These results have been obtained from data on a single herd of relatively high and uniform production and maintained under controlled environmental conditions. Field tests are now under way in many areas to determine, under a variety of conditions, the reliability of evaluation grades for mammary development as a basis for selection.

P34. Effects of udder innunction with diethylstilbestrol on mammary congestion in first-calf heifers. Joseph Meites, R. E. Horwood, E. P. Reineke, C. S. Bryan and E. S. Smiley, Michigan Agr. Expt. Station

In 1st-calf heifers, udder congestion is encountered frequently, with attendant discomfort to the animals and difficulties in management. It is believed that the congestion is caused primarily by a lymphatic and venous stasis and not by filling of the udder with milk. Inasmuch as estrogens have been shown to increase circulation in the mammary gland, it was considered probable that local application of an estrogen to the udder would prove beneficial in alleviating congestion.

On the 1st and 3rd days after calving, the udders of 8 heifers of the college herd were massaged with 200 mg. of diethylstilbestrol dissolved in 10 ml. of corn oil. An equal volume of blank corn oil was rubbed on the udders of 6 control 1st-calf heifers. Daily ratings of udder congestion were made in all heifers and chemical and microscopic examinations were made of the milk.

By the 3rd day postpartum, udder congestion in the diethylstilbestrol-treated heifers was reduced to half, whereas in the controls, udder congestion remained as marked as on the day of calving. Udder congestion continued to be reduced more rapidly in the hormone-treated than in the control heifers during the 2-wk. period of observation. The leucocyte count in the milk also was lowered more rapidly in the diethylstilbestrol-treated heifers. No effects of the hormone on milk production were noted.

P35. Estimation of the thyroxine secretion rate without sacrifice of the animals.[1] G. W. Pipes, C. R. Blincoe and Kuang-Mei Hsieh, Missouri Agr. Expt. Station

The present methods used for the determination of the thyroxine secretion rate require the sacrifice of the animals. For the larger domestic animals such as goats and cows, it is important to develop a method by which repeated measurements may be made during growth, pregnancy and lactation upon individual animals.

Considering the fact that conclusive evidence is available to demonstrate that thyroid secretion rate is governed by the thyroxine level of the blood, it seemed desirable to study the effect of graded thyroxine dosages upon thyroid function, in hopes of devising such a method. Maintaining the thyroxine level of the blood at or in excess of the normal secretion rate should result in minimum function of the thyroid.

In the present study, rats receiving dosages of thyroxine ranging from 0.5–10 μg./100 g. body weight were injected with 10–15 microcurries of I^{131}.

In preliminary experiments, the rate of collection of radioiodine by the thyroid and the formation of protein bound radioiodine (thyroxine?) in the blood indicates that thyroid function reaches a minimum as the thyroxine dosage reaches the normal secretion rate.

[1] Contribution from the Dept. of Dairy Husbandry, Missouri Agricultural Expt. Station, Journal Series no. 1204.

P36. Persistence of different causative organisms in mastitis infections. Lloyd A. Burkey, Cecelia R. Buckner and W. W. Swett, Bureau of Dairy Industry, U.S.D.A.

A study of bovine mastitis in the dairy herds maintained by the Bureau of Dairy Industry at Beltsville, Md., shows that infections by generally recognized mastitis infective organisms occur continuously in spite of symptomatic treatment. Furthermore, recurrent increases in numbers of infections occur irrespective of season or other evident factors. Although the incidences of infection by mildly infective streptococci and hemolytic staphylococci have been reduced during the last 2 yr., there has been an increase in the incidences of coliform and pseudomonad infections.

A study of both new and chronic infections shows that hemolytic staphylococci, *Streptococcus agalactiae,* pseudomonads, and *S. uberis* are often difficult to eliminate by a single treatment and infections by these organisms tend to persist and become established in the udder.

It is apparent from these results that the eradication of the major mastitis organisms in a large herd requires, in addition to therapeutic treatment, extreme vigilance in their detection and improved practices in herd management.

P37. Factors involved in the Whiteside reaction. W. E. Petersen, J. F. Grimmell and I. A. Schipper, Univ. of Minnesota

In a study of the reliability of various tests for mastitis, the Whiteside reaction was found to rate well. By centrifugation it was found that the factors responsible for the reaction resided in the precipitate and not in either the serum or fat. That the white cells *per se* were not responsible for the reaction was proven by negative reaction from concentrated cells from "negative" milk and isolated white cells from blood in healthy and infected cows with leucocytosis when added to normal milk. Exudates from abscesses added to normal milk gave positive reaction, as did expressed juice from either healthy or diseased mammary tissue.

Evidence that the reaction is caused by adsorption of fibrin on the white cells in the milk was obtained by the addition of blood plasma to negative milks reinforced with 1 million or more white cells/ml. Blood serum or plasma with anti-coagulants gave negative reaction when added to such fortified milks, presumably because the fibrin did not adsorb on the cells. Finely divided charcoal plus blood plasma added to cell-free milk serum gave positive reaction.

P38. The influence of soybean hay on reproduction in the rabbit. K. A. Kendall and G. W. Salisbury, Univ. of Illinois

As a consequence of feeding rabbits soybean hay, a syndrome characterized by a reduced number of known pregnancies, small litters, high fetal mortality as shown by numerous partial resorptions, abortions and stillbirths, severe uterine bleeding, hemorrhage in other parts of the body, occasional fetal hemorrhage, prolonged gestation and death of does has been observed. A total of 71 matings resulted in 48 known pregnancies, of which 39 were carried to full term, producing 226 young or 5.8 young/litter.

When alfalfa, lespedeza and timothy were fed, 34 matings resulted in 32 known pregnancies, all of which resulted in litters carried to term, producing 267 young or an average of 8.3 young/litter.

P39. A duodenal fistula for physiological studies in the bovine. G. M. Ward, F. W. Young and C. F. Huffman, Michigan State College

A fistula in the duodenum, approximately two-

thirds the distance from the pylorus to the entrance of the bile duct, was exteriorized immediately dorsal to the costo-chondral articulation of the 11th rib of a 700-lb. Holstein steer. The fistula was closed with a 2-piece plug shaped from Plexiglass. Collection of samples was effected by means of removing the fistula plug, allowing the lumen of that portion of the duodenum extending toward the bile duct opening to empty and then holding a vessel at the lower edge of the fistula to catch the chyme as it was propelled to the fistulous opening by peristaltic waves.

This technique, when combined with the use of a technique involving the use of a natural or artificial inert material as a "marker," offers a means of obtaining information regarding preintestinal digestion, absorption, excretion and synthesis.

P40. Tests with sulphur dioxide for forage preservation. J. B. SHEPHERD, H. G. WISEMAN, R. E. ELY, C. G. MELIN AND C. H. GORDON, Bureau of Dairy Industry; L G. SCHOENLEBER AND L. E. CAMPBELL, Bureau of Plant Industry, Soils and Agriculture Engineering, Agricultural Research Administration; AND W. H. HOSTERMAN, Grain Branch, Production and Marketing Administration, U.S.D.A.

SO_2 was used in a number of forage preservation experiments at Beltsville in 1949. The field treating of freshly cut alfalfa in the swath with 70–80 lb. of SO_2/acre did not increase the drying rate but did increase the rate of carotene loss during field curing. The stand or following growth of the crop was not affected. When injected into the top layer of well-tramped but uncovered silage, SO_2 delayed slightly but did not prevent formation of top spoilage.

SO_2 treatment of undercured baled alfalfa hay slowed down cell respiration for 1 or 2 wk., as judged by temperature measurements, and delayed heating, drying and molding. Experiments with hay in this "dormant" condition were conducted to improve the preservation of carotene and nutrients by barn curing on mow driers.

Baled alfalfa treated with SO_2 and cured on a mow drier without heat had a higher carotene content than similar untreated alfalfa; this effect was greater in uncrushed alfalfa than in field crushed alfalfa.

Two 3-ton lots of chopped alfalfa were cured on mow driers simultaneously without heat. One lot was treated with 25.8 lb. of SO_2, admitted through the main air duct and blown through hay soon after storage. Although the hay was drier than usual when placed on the drier, the SO_2-treated lot showed a preservation of over 5% more dry matter and 20% more carotene than the untreated hay.

P41. A comparison of fecal nitrogen excretion rate, chromium oxide and "chromogen(s)" methods for evaluating forages and roughages. P. G. WOOLFOLK, C. R. RICHARDS, R. W. KAUFMAN, C. M. MARTIN AND J. T. REID, Cornell Univ.

The use of fecal nitrogen as an index of digestibility and dry-matter intake was found to be inaccurate, due to the variability in the rate of nitrogen excretion which was not associated with the level of protein in the forages or roughages fed to calves and lambs. The average and range of fecal nitrogen excretion in grams/100 g. dry-matter intake for calves consuming the following feeds were: barn-cured mixed hay (9.72% protein), 0.72 (0.63–0.81); field-cured mixed hay (9.71% protein), 0.73 (0.71–0.78); mixed hay crop silage (10.85% protein), 0.88 (0.78–0.94); good field-cured mixed hay (11.13% protein), 0.73 (0.70–0.76) and timothy-mixed pasture grass: vegetative stage (16.15% protein), 0.71 (0.69–0.74); boot-to-early-head stage (12.00% protein), 0.66 (0.64–0.72) and full bloom stage (10.41% protein), 0.67 (0.64–0.70). The following values were found for lambs: oven-dried mixed hay (8.38% protein), 0.62 (0.58–0.64); barn-dried mixed hay (9.64% protein), 0.63 (0.59–0.65); field-cured mixed hay (9.41% protein), 0.67 (0.63–0.71) and mixed hay crop silage (11.08% protein), 0.91 (0.76–1.08). Chromium oxide also was found to be inaccurate as a reference substance for determining digestibility due to variation in recovery from the feces. Using a modification of Barnicoat's method of analysis, recovery in trials with calves averaged 99.63% (range 89.32–108.66%); in trials with sheep, 97.11% (range 86.72–109.09%). The level of "chromogen(s)" in individual samples of feces taken at various hours of the day was found to be comparable to that in the composite sample for a complete digestion trial. This indicates the possibility of using individual samples for determining digestibility and consumption following a suitable preliminary period.

P42. A study of the use of chromium oxide and lignin as indicators of digestibility. E. A. KANE, W. C. JACOBSON AND L. A. MOORE, Bureau of Dairy Industry, U.S.D.A.

In order to obtain data on the variation of the percentage of indicators in the feces of cows on ratio technique digestion trials the following experiment was undertaken.

Three cows which had received approximately 15 g. daily of chromium oxide for the previous

90 d. were used. During a 24-hr. period, each individual passage of feces from these cows was mixed and analyzed for dry matter, lignin and chromium oxide.

The ratios of the per cent of the indicator in the feed to the per cent of the indicator in the feces were calculated for each passage of feces from each cow for the 24-hr. period. The averages of the ratios were similar to those calculated for the preceding 9-d. total collection trial.

The range of the variation of these ratios for each of the 3 cows was 0.0516, 0.0866 and 0.0810, respectively, for chromium oxide and 0.0345, 0.0338 and 0.0290 for lignin. These variations are sufficiently large to cause some error in the dry matter digestibility coefficients. The graphs of the chromium oxide ratios of each of the 3 cows followed a similar pattern over the 24-hr. period. The lignin graphs of each cow, while in general agreement with each other, differed from those of the chromium oxide. The results of this investigation show that the time of sampling and the number of samples taken is important in obtaining accurate digestibility coefficients with the ratio technique.

P43. The effect of dosage level and method of administration of DDT on the concentration of DDT in milk. R. E. Ely and L. A. Moore, Bureau of Dairy Industry, and R. H. Carter, H. D. Mann and F. W. Poos, Bureau of Entomology and Plant Quarantine, U.S.D.A.

Crystalline DDT was fed to several milking animals in dosages varying from 50–2000 mg. daily. The material was administered as (a) a soybean oil solution in capsules, (b) crystalline material in capsules and (c) a soybean oil solution mixed in grain. No significant differences in the DDT concentration in the milk occurred when the same dosage level was fed by these 3 methods of administration. Increasing the daily dosage of DDT resulted in comparable increases in the DDT concentration of the milk.

Two crops of hay made from alfalfa previously sprayed with DDT have been fed to 8 milking animals. The daily intake of DDT ranged from 109–727 mg. and progressive increases in the DDT concentration in the milk occurred with increasing intakes. The concentrations of DDT in the milk, however, were more than twice as high as when comparable dosages were fed as crystalline DDT.

When cows were fed DDT in oil solution containing the same proportions of 2 detergents as the material sprayed on alfalfa forage, the concentration of DDT in the milk was similar to that of control animals fed DDT in oil solution without the detergents. This indicates that the detergent used on the treated forage is not responsible for increased absorption and excretion in the milk of DDT fed to dairy cows.

P44. Studies on casein utilization by young calves by use of radioactive tracers. G. P. Lofgreen, Univ. of California

Casein has been tagged with radioactive phosphorus by the injection of lactating cows with inorganic phosphate solution containing P^{32}. The tagged casein was incorporated into purified diets and fed to calves. Studies were made of blood levels of the isotope, its distribution in the body at 24 hr. after bleeding and its excretion in the feces.

The blood levels reached their maximum usually at between 15 and 18 hr. after feeding. There was little difference in the distribution of the isotope in the tissues when it was given in the organic form in casein, fed as inorganic phosphate, or injected directly into the blood stream as inorganic phosphate. Brain tissue consistently contained the lowest concentration of the isotope, while liver consistently was high. The rumen epithelium was higher in concentration than any other part of the digestive tract. By use of the ratio of nitrogen to radioactive phosphorus in the casein and in the feces it has been possible to calculate the metabolic nitrogen excretion of calves while being fed purified milk diets. Using the values thus obtained, the true digestibility of the casein was shown to be between 91 and 95%.

P45. The biological activity of alpha and beta casein-thyroprotein.[1] M. B. Behrens, G. W. Pipes and C. W. Turner, Missouri Agr. Expt. Station

Since the thyroidal activity of iodinated casein results from the iodination of tyrosine and the subsequent coupling of 2 molecules of diiodotyrosine to form thyroxine, it would seem likely that the iodination of α-casein containing 8.1% tyrosine would produce a more active product than could be obtained from β-casein containing 3.2% tyrosine or from whole casein containing 6.3% tyrosine.

In the present investigations, α-casein and casein fractions containing varying amounts of α- and β-casein have been iodinated, and the resulting iodinated casein has been assayed biologically, chemically and by an isotope dilution method employing radioactive iodine.

[1] Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series no. 1207.

P46. Thyroxine content of synthetic thyroprotein as determined by a radioactive isotope dilution

technic. E. P. REINEKE, D. P. WALLACH AND L. F. WOLTERINK, Michigan State College

Previous studies have shown that the thyroidal activity of thyroproteins is related to their *n*-butanol-soluble iodine content. Although thyroxine can be isolated from such preparations in yields of 0.4–0.5%, no direct quantitative measurements have been available on their true thyroxine content. To obtain such a direct measure, a method was devised employing thyroxine labelled with radioactive iodine (I^{131}).

The radioactive thyroxine was prepared by iodination of casein with I^{127} together with a small amount of I^{131}. Subsequent to hydrolysis with $Ba(OH)_2$, the radioactive thyroxine was recovered by crystallization procedures. A known amount of radioactive thyroxine was added to thyroprotein samples to be analyzed prior to their hydrolysis with $Ba(OH)_2$. Thyroxine was recovered and purified by repeated crystallization. The radioactivity of a weighed portion of this thyroxine then was determined, and the thyroxine content of the original sample was computed by calculating the extent of dilution of the radioactive thyroxine originally added by inert thyroxine recovered from the thyroprotein. Eleven thyroprotein samples analyzed contained from 1.13–3.25% apparent thyroxine by the *n*-butanol extraction procedure. By the isotope dilution technique, their true thyroxine content ranged from 0.293–0.753%. The average thyroxine value by the isotope dilution method was 22.9% that obtained by the *n*-butanol extraction method.

P47. The influence of thyroprotein feeding on gains in body weight of dairy calves. RALPH P. REECE, New Jersey Agr. Expt. Station

Thyroprotein was incorporated in dried milk solids supplemented with vitamins (Kaff-A) and fed at various levels to 4-d.-old calves for 10–88 d. Grain was fed *ad libitum* and all the hay that the calves would eat readily was fed twice daily. The feeding of 1 or 2 g. of thyroprotein daily for 88 d. had no significant influence on gains in weight in 6 pairs of calves. The average birth weight of thyroprotein-fed calves was 77 lb. and that of control calves was 80. At 3 mo. the average weight of the thyroprotein-fed calves was 178 lb. and that of the control calves was 173 lb. Three calves received 3 g. of thyroprotein daily for 10–14 d. This level of thyroprotein caused a loss in body weight and at 1 mo. of age the calves were below birth weight. Upon withdrawal of thyroprotein from the ration, the calves were fed whole milk and at 3 mo. their body weights were normal. A Jersey bull calf born in July failed to gain in weight when fed 2 g. of thyroprotein daily for 17 d. The daily feeding of 3 g. of thyroprotein in conjunction with 50 μg. of vitamin B_{12} resulted in a loss in body weight in a Jersey bull calf born in August. Thyroprotein withdrawal and continuation on Kaff-A resulted in marked increases in body weight. The per cent increase in body weight of a bull calf fed 2 g. of thyroprotein daily for 57 d. was 59, whereas that of a calf fed 2 g. of thyroprotein in conjunction with 50 μg. of vitamin B_{12} was 93. The gain in weight of a heifer calf fed 50 μg. of vitamin B_{12} in conjunction with Kaff-A was no greater than that of a calf fed Kaff-A with no supplementation.

P48. The use of x-rays for the detection of rickets in calves. J. W. THOMAS, Bureau of Dairy Industry, U.S.D.A.

Accurate diagnosis of rickets in calves has not been possible because the physical and/or chemical methods employed have not been sufficiently sensitive and applicable. This has been especially true under field conditions where obvious symptoms of rickets rarely occur.

By the use of x-rays, following the procedure outlined by Bechdel *et al.*, it was found that the width of the epiphyseal cartilage of the ulna of normal calves follows a definite pattern. From birth to 8 mo. of age it gradually ossified and closed. The roentgenogram was taken of the left ulna at about a 75° angle from front view. The results of this technique were correlated with studies made on plasma Ca, P and phosphatase and with bone ash of over 75 calves. This technique offered an excellent means of detecting rickets and it can be used advantageously where it is difficult to obtain data by the usual methods.

This technique indicated that calves allowed limited access to sunshine and fed barn-dried alfalfa or alfalfa silage as their sole roughage were free from rickets.

Another group of calves was kept in a well-lighted barn and fed skim or whole milk, shark liver oil and grain. In mid-December, when they were 68–118 d. of age, they showed evidence of rickets as shown by roentgenograms. Only 1 calf showed gross symptoms of rickets. Rapid closure was effected by feeding viosterol; slow closure by limited winter sunshine.

P49. An approach to the problem of the etiology of ketosis in dairy cows. B. C. HATZIOLOS AND J. C. SHAW, Univ. of Maryland

Some of the more important histopathological findings observed in a significant per cent of cases have been cystic degenerations, atrophy and fatty degeneration of the anterior lobe of the pituitary and enlarged adrenals with degenerative areas

in the outer zones. An adrenal with polymorphonuclear infiltration in the zona fasticulata and another with an abnormal growth in the cortex have been observed. The response of ketotic cows to adrenal cortical extracts and one case of response to cortisone also indicates that the pituitary-adrenal system is involved. Numerous abnormalities have been observed, such as inflammation or congestion of the abomasum and small intestine, ulcer, metritis and lipofibroma around the kidney. It is considered significant that in practically all of the cases the cows exhibit typical symptoms of ketosis and respond to intravenous injections of glucose.

A tentative proposal is made that ketosis in cows is basically a disease of the so-called adaptation syndrome, brought on by various stresses during a period when the organism is overworked.

P50. Kentucky 31 fescue as a dairy pasture in northern Ohio. AVERY D. PRATT AND JAMES L. HAYNES, Ohio Agr. Expt. Station

Kentucky 31 fescue was seeded with alfalfa and ladino clover in 1948 and first grazed in 1949. Good stands of ladino and Kentucky 31 resulted with only a small admixture of alfalfa. Jersey heifers made from 0.2–0.4 lb. gain in body weight daily while grazing it, as compared to 1.7 lb. when grazing bluegrass and ladino or meadow crop mixtures. Milking cows in reversal experiments ate more hay, produced less milk and lost more weight when grazing Kentucky 31 and ladino than when grazing bluegrass and ladino. Again in comparison with meadow fescue, Birdsfoot trefoil and ladino, the Kentucky 31 and ladino gave similar results. A reversal experiment with 1 group of cows on Kentucky 31 and ladino and the other in dry lot resulted in slightly higher milk production when in dry lot. Silage consumption was nearly the same for both lots. Those in dry lot ate more hay and gained more in body weight.

Ohio farmers are advised not to seed Kentucky 31 fescue until more favorable results are obtained.

P51. Fourteen years with supplementary pastures. N. R. THOMPSON AND C. W. HOLDWAY, Virginia Agr. Expt. Station

Various legumes and grasses were grown singly and in combinations over a period of 14 yr. and grazed by dairy cattle. Plots of bluegrass were grazed the 1st 5 yr. Estimated total digestible nutrient yields/acre/season included 2,186 lb. for alfalfa-bromegrass, 2,019 for alfalfa-orchard grass, 1,946 for ladino clover, 1,678 for Korean lespedeza-orchard grass, 1,569 for bluegrass, 1,424 for crimson clover-Abruzzi rye-Italian rye grass, 1,368 for Korean lespedeza-orchard grass-sweet clover and 986 for Korean lespedeza-bromegrass. Average production in pounds of 4% fat-corrected milk/cow/day on the above pastures was 33.1, 27.1, 30.8, 29.3, 21.7, 22.3, 0, and 28.7, respectively. Values for milk production are not strictly comparable, since they were made by cows in various stages of lactation and over a number of years, but may be assumed to indicate some possibilities of the various pastures for milk production.

Some pastures were available for grazing throughout the season, others for only short periods. Alfalfa-orchard grass and alfalfa-bromegrass were available continuously from late April to late Sept. Crimson clover-Italian rye grass and crimson clover-Abruzzi rye were available principally in April and May and occasionally in Oct. and Nov. Sudan grass was available for grazing in July and Aug. Several pastures showed peaks in nutrient production in May and June.

P52. The effects of partial replacement of alfalfa hay with concentrates. K. A. KENDALL AND R. D. ENGBERSON, Univ. of Illinois

Four groups of 2 cows each were fed alfalfa hay and salt for a period of 8 wk. In the 2-wk. experimental period that followed, each cow within a pair was fed a reduced amount of alfalfa and 1 other nutrient source. The other nutrient source was either 7 lb. ground corn, 7 lb. starch, 7 oz. corn oil or 7 oz. coconut oil replacing in the daily feed allowance 11, 12.5, 2.5, or 2.5 lb. of alfalfa hay, respectively. T.D.N. intakes for the experimental period were from 4–8% less than those of the preceding 2-wk. basal period of alfalfa hay feeding.

The per cent increase (+) or decrease (−) in pounds of 4% F.C.M. produced per pound of T.D.N. consumed in the experimental period, as compared with that in the basal period, was for corn, +6.0 and −47.0; starch, −5.2 and −14.9; corn oil, 0 and +5.0; and coconut oil, +8.8 and +9.0.

P53. Efficiency of silage and extra grain feeding for maintaining summer milk production. DWIGHT M. SEATH AND RALPH F. ELLIOT, Kentucky Agr. Expt. Station

Feeding either corn silage or an extra amount of grain mixture to Holstein and Jersey milk cows on pasture from Aug. 1 to Sept. 11, 1949, aided in preventing the late summer decline in milk production. The benefits probably were not large enough to be economically profitable, although some of the benefits that might have resulted were nullified by the presence of better-than-average grazing.

In terms of 4% FCM, the daily milk production per cow for the 42-d. experimental period, when compared to that for the 14-d. preliminary period, declined 2.73 lb. for cows receiving the check ration, declined 1.25 lb. for those receiving the silage as supplement, and increased 1.12 lb. for cows receiving the extra allowance of the grain mixture.

Cows fed the check ration received a 16% grain mixture at the rate of 0.6 lb. for each 1 lb. milk in excess of 10 lb. daily for Jerseys and 0.4 lb. for each 1 lb. milk in excess of 15 lb. for Holsteins. Those receiving silage were fed grain, as were the check group, but in addition were allowed all the silage they would clean up. Their average daily consumption was 15.4 lb./cow and this contributed 18% of the calculated total digestible nutrients (TDN) required by the cows.

Cows receiving extra amounts of the grain mixture as a supplement consumed 2.7 lb. of grain/cow daily in addition to the amounts required when calculated as for the check group. In terms of TDN, this was 17.3% of their calculated requirements.

Body weight data for the cows during the experimental period showed that those in the check group gained 36 lb./cow, those receiving silage gained 70 lb., and those receiving extra grain gained an average of 82 lb./cow.

P54. Rates of grain feeding for dairy heifers on temporary and permanent winter pasture. S. H. Morrison and J. F. Deal, Univ. of Georgia

Twelve purebred Jersey heifers ranging in age from 8–12 mo. of age were divided into 3 outcome groups of 4 animals each. All groups received pasture and U. S. no. 1 lespedeza hay, free choice. Two groups were on temporary pastures comprised of a mixture including Italian rye grass, oats and crimson clover. The 3rd group was on a permanent winter pasture comprised of a mixture of ladino clover and fescue (Alta and Ky. 31).

One control heifer in each group was fed 2 lb. of grain daily (12% total protein) throughout the entire experiment. Each of the other 3 animals in each group received 1 of the following amounts of grain in a Latin square design during each experimental period of 5 wk.: 0, 2, or 4 lb. There was a transition period of 1 wk. between the experimental periods.

Data will be presented on the actual hay consumption, grain consumption, weights and height at withers of the heifers on the 2 types of pasture. Tentative recommendations will be made for grain feeding rates on both types of pasture.

P55. Kudzu and fescue-ladino clover silages for dairy cows. W. A. King and J. P. LaMaster, So. Carolina Agr. Expt. Station

Kudzu and fescue-ladino clover silages were compared to corn silage in a 16-wk. continuous feeding trial. The kudzu was ensiled in Sept., 1949, with 61 lb. of molasses added/ton of green material. The fescue-ladino clover (70–30%) silage was ensiled in May with 65 lb. of molasses added/ton. Eight cows in each of 3 groups were fed the respective silages, Kobe lespedeza hay and a concentrate mixture. The daily silage consumption per cow averaged 45.6 lb. corn silage, 44.4 lb. kudzu silage and 47.8 lb. fescue-ladino clover silage for the respective groups. The fescue-ladino clover silage was as palatable as the corn silage, whereas the kudzu was less palatable.

The average daily milk production (4% F.C.M.) per cow was 34.9 lb. for the corn silage group, 35.1 lb. for the kudzu silage group and 34.6 lb. for the fescue-ladino clover silage group. The gains in live weight per cow averaged 48, 8 and 38 lb., respectively.

The carotene content of the corn silage averaged 62 p.p.m. of dry matter; the kudzu silage, 166 p.p.m.; and the fescue-ladino clover silage, 240 p.p.m.

P56. Farm grains vs. a medium protein concentrate mixture for cows. K. E. Gardner, Univ. of Illinois

Thirteen cows of the 5 major dairy breeds received, over an 18-wk. period, a 9.5% total protein concentrate composed of 60% corn-and-cob meal, 37.5% ground oats, salt and additional bone meal. A similar control group received added soybean oilmeal and linseed oilmeal to provide a 14% protein mixture. Medium alfalfa hay and corn silage were fed at rates of 1 and 3 lb., respectively,/100 lb. body weight daily. Concentrates were fed at the middle rate recommended in the grain feeding table shown by Morrison. The cows were classed as medium producers, since none exceeded 45 lb. FCM daily.

The low-protein cows averaged 27.11 lb. FCM daily and the medium protein group, 26.45 lb. Analysis of variance showed that this difference was not statistically significant.

P57. The relative palatability of expeller and extracted linseed meal in dairy cow rations. N. N. Allen, Univ. of Wisconsin

Using 16 cows, a palatability test was run comparing a 34% protein, 4.0% fat expeller meal with a 37% protein and 0.5% fat extracted meal. The test rations contained 300 parts each of oats, corn, and linseed meal and 150 parts of wheat bran. During a preliminary period, 8 cows were fed the feed containing expeller meal,

while the others got the feed containing extracted meal. During the 10-d. test period, using a divided feed box, they received ½ of their usual allotment of concentrate in each form, having free choice. They were observed closely for any evidence of preference. Of the 160 feedings to the cows conditioned to the expeller meal, in 14 there was some evidence of preference for the expeller and in 8 for the extracted meal. Of those 160 feedings to cows conditioned to extracted meal, in 2 cases there was evidence of preference for expeller and in 5 for extracted meal. Of the total 320 feedings, in 16 there was evidence of preference for expeller and in 13 for extracted meal. In no case was there any pronounced objection to either feed and, in most cases, the evidence of preference was slight. The conclusions were that there is no practical difference in palatability of the expeller and extracted meals when used in a dairy cow concentrate at the level of 28%.

P58. Preliminary observations on the effects of nutrition on the quality and quantity of bovine semen. H. H. Olson, W. E. Petersen, T. W. Gullickson and J. N. Cummings, Univ. of Minnesota

One set of identical triplet bulls, *T*, *D* and *H* were placed on 3 levels of nutrition. *T* was fed 30% below normal, *D* was fed normally and *H* fed 30% above normal. *D* was used as the guide for *T* and *H*. Two ejaculates were collected from each bull at weekly intervals during an 8-mo. period. These samples were tested for quality and quantity by the following tests: volume, density, total number of sperm per ejaculate, motility, methylene blue reduction and abnormal sperm.

T produced the smallest volume of semen, whereas *H* produced the largest volume. The second ejaculates were larger than the first. Density tests indicated no real differences between the bulls. First ejaculates tended to have a greater density than the second. *T* produced the lowest number of sperm per ejaculate, but little difference was found between *D* and *H*. More sperm were found in the second ejaculates than the first. *T* was found to have the poorest motility and little difference was noted between *D* and *H*. First ejaculates were found to have the best motility ratings. The methylene blue reduction test indicated *T* as producing the poorest semen and *D* the best semen; *H* was found to be just below the reduction time of *D*. The first ejaculates were found better than the second. There was no marked difference between the bulls on number of abnormal sperm produced. However, *T* produced the most abnormal sperm and *H* the least. The second ejaculates contained more abnormals than the first. *H* exhibited the most libido and *T* the least.

The results under the conditions of this experiment indicate that a bull fed a below normal ration (T) produces semen of poorer quality and smaller quantity than bulls fed normal (D) or above normal (H) rations.

P59. A preliminary report on the effect of the site of semen deposition on fertility in artificial insemination. G. W. Salisbury and N. L. VanDemark, Univ. of Illinois

The results of 2 limited though carefully controlled experiments involving 936 cows suggested that cervical deposition of semen was as satisfactory as deposition either into the body or the horns of the uterus. The inseminating catheter was guided into the female reproductive tract by manipulation of the tract through the rectal wall. The conception rate was much higher for insemination into the cervix than had been reported for other experiments in which cervical insemination was accomplished by aid of a speculum.

To obtain more data on the appropriate site of semen deposition, an experiment was conducted involving 18 technicians of the Northern Illinois Breeding Association, 3 time periods of 1 mo. each, and insemination into the cervix, into the body of the uterus, or into each of the uterine horns. Six 3×3 Latin squares made up the design and several thousand cows were inseminated. Preliminary and as yet inconclusive results at the time of writing indicate that there is no appreciable advantage to be gained by insemination beyond the cervix.

P60. Spermatozoan transport in the reproductive tract of the cow. N. L. VanDemark and A. N. Moeller, Univ. of Illinois

Investigations are being carried out to determine the minimum time required for spermatozoa to reach the ovarian portion of the oviduct after deposition in the cervix of the cow by artificial insemination. Ten cows were slaughtered at various intervals after insemination, and the uterus and oviducts were clamped off at various locations. While carefully controlling the temperature of the tract, fluid samples were withdrawn from each isolated location by means of a syringe and needle and examined microscopically for the presence of spermatozoa. The interval from insemination to clamping off after slaughter has been gradually diminished from 140 min. down to 30. Spermatozoa of varying degrees of activity have been used and were found in the ovarian portion of the oviducts at the shortest interval studied. To eliminate the possibility

that spermatozoan transport in the first of this study was a result of the slaughtering procedure, the right horn and oviduct of each of a second series of cows were clamped off before slaughter through a surgical opening in the flank. Using this technique, spermatozoa have been found in the ovarian portion of the oviduct when the interval between insemination and clamping off was 11 min.

P61. The effect of sterile copulation on the time of ovulation in dairy heifers. GERMAIN G. MARION, VEARL R. SMITH, THOMAS E. WILEY AND GEORGE R. BARRETT, Univ. of Wisconsin

The effect of sterile copulation on the time of ovulation in 25 dairy heifers was determined. The time of ovulation was established from the time when the heifer was observed to go out of estrous. Each heifer was observed through 4 clinically normal estrous periods which were alternately designated as control and experimental periods; thus, each animal served as her own control. During each of the 2 experimental periods, the heifer was serviced once by a vasectomized bull.

The average time interval between the end of heat and time of ovulation following non-service or control periods was 9.91 hr., while this interval for experimental periods was 7.73 hr. Statistical analysis showed this difference of 2.18 hr. to be highly significant.

P62. Measuring reproductive efficiency in dairy cattle.[1] F. A. BUSCHNER, R. E. JOHNSON, C. I. BLISS AND A. A. SPIELMAN, Storrs Agr. Expt. Station, Univ. of Connecticut.

The breeding records from the University of Connecticut dairy herd were analyzed statistically in order to devise a measure of reproductive efficiency which takes into account the various measurable factors affecting the reproductive life of a cow. The effect of 4 factors upon the age at 3rd calving was studied, namely, age in days at first breeding, the interval from first breeding to conception for the first reproductive cycle, interval from calving to first breeding for the second and third reproductive cycles and interval from first breeding to conception for the second and third reproductive cycles. Partial regression coefficients for each factor were calculated for each breed separately and used to compute an estimated age at 3rd calving. More than 99% of the variability in age at 3rd calving was accounted for by these partial regression equations, although in the aggregate they represented only ½ of the total period. A new score for reproductive efficiency then was based upon the age in days at 3rd calving. Cows reaching this age before the herd average are the more efficient reproducers, if the effect of abortions is excluded. The new measure, in units of the standard deviation from the herd mean, shows an animal's relative position in the herd and facilitates the comparison of animals from different herds. This measure can be estimated with practicable reliability as soon as a heifer is safely in calf from partial regression equations based upon age at first breeding and the interval from first breeding to conception.

[1] Supported in part by funds provided under Section 9B3 and 10A, Research and Marketing Act, 1946, in cooperation with the Bureau of Dairy Industry.

P63. The sex ratio in calves resulting from artificial insemination. K. E. GARDNER,[1] Univ. of Illinois

This study was prompted in part by a recent paper reporting that the sex ratio in rats was affected by the length of the interval between ovulation and insemination. In the artificial insemination of dairy cattle the cow probably is bred later in the heat period than would be the case in natural breeding. This should result in a shorter-than-normal time interval between insemination and ovulation, since the cow normally ovulates postestrus.

A sex ratio at birth of 100 females to 105.89 males (917 males to 866 females) was found in a survey covering 1,783 single-born Holstein calves resulting from artificial insemination. The data were obtained in studies conducted in 26 herds which kept accurate and complete breeding and calving records. This sex ratio is very similar to the ratio of 100 females to 106.2 males obtained by Johansson in his studies of 125,000 calf births from natural service in Scandinavian herds. A summary of the twins born in these Illinois herds showed that 9 calvings produced male twins, 21 calvings produced female twins, and 27 calvings produced mixed twins, making a total of 1897 calves in the study.

[1] The assistance of L. S. Zuckerman is gratefully acknowledged.

P64. Artificial breeding in Alaska and the effect of extra light during the short winter days. WILLIAM J. SWEETMAN, Alaska Expt. Station, Palmer

In Matanuska Valley the possible sunlight on Dec. 21 is 5 hr. and 28 min. In the winter of 1948–49 a number of farmers gave their cows 14 hr. of light/day. The others received only the natural light plus what was needed to do chores. This amounted to an average of 8 hr. or less/day. Of 111 cows receiving additional light bred from Oct. 1, 1948, to May 31, 1949, the conception rate was 53.6% and the conception

rate of 93 cows in the natural light group was 48.6%. Services per conception were 1.86 in the additional light group and 2.05 in the natural light group. This is a significant difference.

The seasonal percentages of non-returns was as follows: fall of 1948, 55.2%; winter of 1948, 44.0%; spring of 1949, 55.0%; summer of 1949, 48.3%; and fall of 1949, 54.6%.

Of 1178 inseminations from Aug., 1948, to Jan., 1950, there were 584 1st services, 320 2nd services, 157 3rd services, 80 4th services and 34 5th services. There were 3 services beyond the 5th. The corresponding percentages of non-returns were 50.7, 57.2, 56.1, 50.0 and 29.4. The over-all percentage of non-returns was 52.4.

P65. Nutritive value of crops and cows' milk as affected by soil fertility. II. The amino acid composition of colostra and milk.[1] C. W. Duncan, K. M. Dunn and Gertrude I. Watson, Michigan Agr. Expt. Station

An experiment was initiated in 1945 to study the characteristics of certain plant species grown on natural soils seriously depleted of mineral nutrients and on the same kind of soil with the addition of adequate amounts of lime and mineral fertilizers. The feeds grown on the fertilized and depleted soils are being fed to 2 groups of cows as their sole ration. The composition of the crops, milk and blood has been determined at regular intervals since the beginning of the experiment.

The amino acid content of the colostra, 60-d. composited milk and terminal milk samples of the 2 groups of cows has been determined by microbiological methods for arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. These data have been collected from the 1st through the 4th lactation period. No difference has been obtained in the amino acid pattern of either group for the 0-hr. or 24-hr. colostrum samples or between the Jersey or Holstein breeds, with the exception of arginine, which tends to be higher in the colostrum of the cows receiving the unfertilized feed. The threonine content of the 60-d. composited milk and terminal milk samples was markedly lower than the colostrum in both the Jersey and Holstein breeds, but this phenomenon can not be attributed to the feeds.

With the exceptions noted, there is no essential difference in the amino acid composition of the colostra and milk of the 2 groups of cows that can be attributed to feeds grown on depleted soil or on highly fertilized soil of the same type.

[1] This work was supported in part by a grant from the National Dairy Council on behalf of the American Dairy Association.

P66. Dairy cow stall studies. I. D. Porterfield, George Hyatt, Jr., D. P. Brown and A. D. Longhouse, West Virginia Univ.

A comparison of Holstein cows kept in 2 types of stalls was made at West Virginia University. There were 7 "tie chain" stalls each 66 in. long and 42 in. wide and 7 "comfort" stalls 84 by 49 in. with an adjustable "cross bar" in each one. Data were collected between Oct. and May in 1947, 1948, 1949 and 1950.

Eight cows in "comfort" stalls produced 2–12 lb. more milk (2X M.E. basis) daily for a minimum of 14 wk. than when they were in "tie chain" stalls. In 2 trials, 5 of 7 cows remained cleaner in the "comfort" stalls than they did in "tie chain" stalls. Difference in daily requirements for bedding for each type of stall was not statistically significant. Nine observations showed no significant difference in time needed to clean the 2 types of stalls. No cases of mastitis have been encountered.

Observations for leg injuries were made on the 14 cows 3 times each week. Two mo. after cows were stabled 7 in the "tie chain" stalls showed injuries as compared with 1 in "comfort" stalls.

Cows in the "tie chain" stalls spent 8.8 hr./24-hr. period lying down as compared to 10.2 hr. for cows in the "comfort" stalls. The difference was statistically significant.

P67. Calf losses from disease. H. P. Davis, Univ. of Nebraska

The dairy herd at the University of Nebraska has had an unusually continuous system of management, with only 3 persons responsible for the management practices in 44 yr. In a study of the births and final disposal of calves from 4 dairy breeds, Holstein, Jersey, Ayrshire and Guernsey, the abortions, live births, sex ratio, twins and losses from disease were tabulated and reduced to percentages. Altogether, 2,362 calvings were involved with a total of 2,428 calves being born, with a sex ratio of 51.48% males to 48.52% females. There were 149 abortions or 6.31% of the calvings. Sixty-six twins were born or 2.79% of calvings. Altogether, 2,166 calves were born alive or 89.21% of calvings. Disease losses were divided into digestive, respiratory, infectious and other causes.

Thus, there was a total of 14.77% loss from disease during the first 2 yr. of life. From 1 to 3 mo. the losses due to these causes were 1.80, 3.55, 1.66 and 5.12%, respectively, from 4 to 6 mo. they were 0.37, 0.88, 0.23 and 0.23% respectively, and from 7 to 23 mo. they were 0.18, 0.23, 0.23 and 0.28%, respectively.

P68. Relation of production records on cows and efficient management of the dairy farm. LEO R. FRYMAN, Univ. of Illinois

Using 3-yr. records from 65 dairy farms in northeastern Illinois, 5 factors were found to have a significant influence on both the rate earned on the total investment in the farm business and net earnings per acre. These 5 factors are: returns for each $100 spent for feed for dairy cattle, level of production of the cows in the herd, size of the herd, labor costs per cow and crop returns on tillable land. Farms which had tested in a DHIA for 10 or more yr. were compared with similar farms which had not tested in DHIA. The 2 groups of farms were similar as to crop returns on tillable land, labor costs per cow and size of the herd.

Herds tested in DHIA had a higher milk and butterfat production, higher returns above feed costs and higher returns for each $100 spent for feed for dairy cattle than non-DHIA herds. They also had a higher rate earned on investment, higher net earnings per acre and higher operator's earnings.

P69. Relation of gestation to body weights of cows on long-time feeding trials. R. B. BECKER, P. T. DIX ARNOLD AND SIDNEY P. MARSHALL, Florida Agr. Expt. Station

Eight non-gravid uteruses (severed at the *os uterus*) of Jersey cows averaged 1.4 lb. Thirty-five Jersey, 1 Guernsey and 1 Jersey-Guernsey fetuses from 32 to 279 d. in gestation, with accompanying placentae, fluids and empty uterus weights, may serve as a basis for computing corrections in weights of Jersey cows on long-time feeding trials. Changes in weight of uterus and contents were negligible up to past 60 d. in gestation. From 90 d. onward, they amounted to 5, 12, 22, 31, 43, 75 and 110 lb. at progressive 30-d. intervals, including the 270th day. At full term, the weight increase attributable to gestation amounted to 122.2 lb., assuming a 55 lb. calf, 15.8 lb. placenta, 38.2 lb. of fluids and allowing 14.6 lb. for involution of the uterus after calving.

Weight corrections would minimize error that arises when gross weight changes of cows advancing in gestation are computed at 3.53 and 2.73 lb. of total digestible nutrients/lb. of body gain or loss, respectively. Such corrections also would reduce a source of serious error arising from attempts to maintain "constant body weight" by adjusting concentrate offerings to pregnant cows on long-time feeding trials.

P70. The influence of oestrum on weights of Holstein and Jersey heifers. H. B. MORRISON, Kentucky Agr. Expt. Station

Heifers weighed 3 consecutive days on a pasture experiment usually weighed less on the day they were in heat. Records revealed 241 instances in which a heifer was weighed when in heat. Of these, 80 were in heat on the 3rd, 91 on the 2nd, and 70 on the 1st d. weighed. Of 171 instances where weights were available the day before and the day in heat, the average decrease in weight was 19.5 lb. The weight differences varied from a drop of 66 lb. to an increase of 20 lb., and standard deviation was ± 13.83 lb. Of 171 instances, 160 (93.6%) weighed less on the day in heat, 1 (0.6%) weighed the same and 10 (5.8%) weighed more. Of 161 instances where weights were available for the day in heat and the day after, the average increase in weight the day after heat was 16.8 lb. The differences in weight ranged from a decrease of 26 lb. to an increase of 70 lb. and showed a standard deviation of ± 16.96 lb. An increase in weight the day following heat occurred in 135 cases (83.8%), in 4 cases (2.5%) the heifers weighed the same and in 22 cases (13.7%) the heifers weighed less on the day following than on the day in heat. The average weight of the heifers on the day in heat was 625 lb.

P71. The effect of a combination of penicillin, streptomycin and sulfanilamide upon the fertility of bull semen. J. O. ALMQUIST AND P. W. PRINCE, Pennsylvania State College

A field trial was conducted at the First Pennsylvania Artificial Breeding Cooperative, utilizing semen from all bulls in active service to determine the effect of penicillin, streptomycin and sulfanilamide upon fertility. Three egg yolk-citrate diluters containing: (a) 1,000 μg. streptomycin sulphate/ml., (b) 1,000 units penicillin G plus 1,000 μg. streptomycin/ml. and (c) 1,000 units penicillin G plus 1,000 μg. streptomycin plus 3 mg. sulfanilmide/ml. were compared with yolk-citrate diluter containing 3 mg. sulfanilamide/ml. (control). Each semen sample was diluted with yolk-citrate and divided into 2 equal portions. One-half received 3 mg./ml. sulfanilamide, while the other half received 1 of the above 3 treatments. The results were based on 6-mo. non-returns involving 296 ejaculates used on a total of 11,715 first and second service cows. The over-all results showed significant mean increases of 5 percentage units for diluters *a* and *c* over the control diluter, while diluter *b* showed an insignificant increase of 2 percentage units. Further analysis, however, revealed that the effectiveness of these diluters was dependent upon the relative fertility levels of the bulls. When divided into high and low fertility groups (based on the average non-return rate for the control

diluter), diluter *b* now produced a significant increase of 7 percentage units among the low fertility bulls, while diluters *a* and *c* brought about highly significant increases of 11 percentage units. On the other hand, in the high fertility group no significant difference was found between the control diluter and diluters *a*, *b* or *c*.

P72. Bull semen toxicity of various salts, brands, and lots of penicillin, streptomycin, aureomycin and chloromycetin. JAMES G. SYKES AND JOHN P. MIXNER, New Jersey Agr. Expt. Station

Two salts including 4 brands (2 lots each) of penicillin, 3 salts including 3 brands (2 lots each) of streptomycin, 2 salts of aureomycin and chloromycetin were tested for their semen toxicity. Six dosage levels of each antibiotic were added to diluted semen which was stored at 5° C. for a 15-d. period. The criterion of toxicity used was the estimated per cent of motile spermatozoa on days 5, 10 and 15.

No differences in toxicity could be demonstrated between the sodium and potassium salts of penicillin or among any of the 4 brands or 8 lots of penicillin studied. Streptomycin in the form of the sulfate, hydrochloride and calcium chloride complex represented the 3 brands and 6 lots studied. Again, significant toxicity differences could not be demonstrated among any of these salts (or brands) and lots of streptomycin. No difference was found between the relative toxicity of aureomycin base and the hydrochloride. However, aureomycin (base or hydrochloride) is relatively toxic to spermatozoa as the initial toxic level is in the general range of 125 to 250 μg./ml. of diluted semen. Chloromycetin would seem to have a relatively low level of toxicity, as the initial toxic levels seemed to be between one and 2 mg./ml. of diluted semen.

P73. The influence of aureomycin upon the livability and bacterial content of bull semen. R. M. MYERS, J. O. ALMQUIST AND P. W. PRINCE, Pennsylvania State College

Six levels of aureomycin hydrochloride (Lederle) ranging from 50–1,000 μg./ml. of diluted semen were studied, using 19 semen samples. All levels caused significant toxic effects on motility when compared with portions of the same semen samples containing no aureomycin or 1,000 units each/ml. of penicillin G (Pfizer) and streptomycin sulphate (Pfizer). Bacterial plate counts of 18 semen samples were made after 0, 4, 8 and 16 d. of storage at 4.5° C. Concentrations of aureomycin from 100–1,000 μg./ml. brought about effective control of bacterial growth. However, the 50 μg. level was not as efficient as the higher concentrations. The antibacterial activity of 1,000 μg. of aureomycin/ml. compared favorably with the combination of 1,000 units each/ml. of penicillin and streptomycin. Assays showed that aureomycin was relatively stable in diluted semen stored up to 16 d. at 4.5° C. Diluted semen samples assayed after 8 and 16 d. of storage at room temperature showed a marked decrease in aureomycin activity.

P74. Hyaluronidase and fertility of dairy bull semen. JAMES E. JOHNSTON AND JOHN P. MIXNER, New Jersey Agr. Expt. Station

It is believed that hyaluronidase is an enzyme which acts in the physiological manner to allow spermatozoa to make contact with the ovum. The purpose of this study was to determine whether a significant relationship existed between the hyaluronidase content and fertility of dairy bull semen diluted at a rate of less than 1: 100.

Hyaluronidase assays on semen were made within 1 hr. of ejaculation and after incubation for 24 hr. at 37° C. under toluene. Fertility estimates were based on 60- to 90-d. non-returns to 1st and 2nd service breedings and include the following 3 categories: (a) 82 semen samples from 21 bulls to which 10–19 breedings were made/sample; (b) 105 semen samples from 18 bulls to which 20 or more breedings were made/sample; and (c) a total of 187 semen samples from 24 bulls to which 10 or more breedings were made/sample for a total of 4,657 breedings with average % N.R. of 66.1.

Data on initial and 24-hr. hyaluronidase levels were correlated with data on fertility in each group on a "total," "between bull" and "within bull" basis. No correlations secured had statistical signficance and it may be concluded that no significant relationship exists between the hyaluronidase and fertility levels of semen from bulls of relatively high fertility when the semen is diluted at a rate of 1: 100 or less.

P75. A comparison of two streptomycin compounds used in diluted bull semen. H. L. EASTERBROOKS, P. HELLER, W. N. PLASTRIDGE, E. L. JUNGHERR AND F. I. ELLIOTT, Storrs (Conn.) Agr. Expt. Station

The purpose of this study was to test dihydro (DH) streptomycin sulfate and streptomycin calcium chloride complex (CCC) for evidence of incompatibility with semen diluter buffers and for their comparative value in producing increased fertility rates. Graduated concentrations of the 2 streptomycins were added to various concentrations of citrate and phosphate buffers. Visible precipitation occurred in all tubes containing phosphate buffers to which streptomycin CCC in excess of 100 units (μg.)/ml. had been added.

Ten ejaculates used by the Connecticut Artificial Breeding Association over a period of 5 consecutive weekends in Oct. and Nov., 1949, were subjected to a split sample study involving the 2 drugs at a level of 500 units/ml. of diluted semen. Comparisons were based on 60- to 90-day non-return (N.R.) to 1st service percentages. Semen treated with DH streptomycin was used to inseminate 394 cows with a % N.R. of 72.34, as compared to 68.03 for semen containing streptomycin CCC used on 398 cows. Although this 4.25% N.R. increase for the DH streptomycin treated semen was not statistically significant ($P < 0.20$), it appears that it may be the drug of choice when streptomycin is added to semen diluters.

P76. Results of breeding dairy cows with egg yolk citrate and Ortho semen diluters. VICTOR HURST, South Carolina Agr. Expt. Station

The Clemson Agricultural College bull stud supplies diluted semen for approximately 400 breeding females in the College herd and also for 15,000 cows in 12 cooperating artificial breeding units in South Carolina. During the past 1.5 yr. these cows have been bred with semen extended in a diluter composed of 40% egg yolk and 60% 0.1 *M* sodium citrate. Although results have been variable, monthly average conception rates of 69% have been obtained on 60- to 90-day non-returns to 1st services.

This diluter of 40% egg yolk and 60% sodium citrate has been compared with a 20% egg yolk and 80% sodium citrate diluter and with the liquid Ortho semen diluter. In the College herd, based on actual calvings of 55 females, the following conception rates to 1st services were observed: 40% egg yolk showed 68%, 20% egg yolk showed 75%, and Ortho showed 60%. This work was followed by field trials in the county associations. Based on 65-day non-returns to 537 1st services, 40% egg yolk gave 66%, 20% egg yolk gave 61% and Ortho gave 62%.

MANUFACTURING SECTION

M1. Ion exchange as a means of varying the salt constituents of milk. H. S. HALLER AND A. G. MORIN, Bureau of Dairy Industry, U.S.D.A.

Milk can be modified by treatment with various types of organic ion exchange materials to produce certain desired characteristics. The type and extent of modification can be controlled at will by using the proper exchangers or combination of exchangers and by the proper regeneration of these exchangers.

An organic cation exchanger regenerated to a 99: 1 Na-H ratio produced a soft curd milk of normal pH with one treatment. The amounts of Ca and other cations removed depended on the time of treatment and the milk-to-exchanger ratio. This milk retained all of its citrates, phosphates and chlorides.

Treatment with a cation exchanger regenerated to a 93: 7 Na-H ratio, followed by treatment with an organic anion exchanger, removed up to appromixately 20% of the citrates, phosphates and chlorides in addition to Ca and other cations. The ratio of cations to anions removed was controlled by using a cation exchanger regenerated with a Na percentage ranging between 93–99, followed by treatment with an anion exchanger.

Treatment with a strong anion exchanger, followed by careful treatment with a small amount of cation exchanger regenerated 100% in the hydrogen cycle, removed citrates and chlorides and small amounts of Ca and other cations, but no phosphates.

M2. Isolation of the non-casein proteins of milk. A. R. KEMP, B. C. JOHNSON AND A. M. SWANSON, Univ. of Wisconsin

Many properties of milk have been attributed to the non-casein proteins. Only limited quantities of certain of these proteins have been isolated for study.

In the present study 4 fractions of non-casein proteins are obtained by control of salt concentration, pH and temperature. Casein is precipitated by acid at pH 4.5, 10° C.; non-casein proteins are isolated as follows: Fraction 1—salt saturation at pH 6.0–6.2, 2° C. The precipitate is allowed to settle, supernatant siphoned off and the precipitate washed 3 times with saturated salt water. Fraction 2—the precipitate formed with 14 g. salt/100 ml. solution at pH 3.5 or lower and temperatures up to 25° C. Fraction 3—the filtrate from fraction 2.

Curves were obtained of protein precipitation by changes in pH from 7.0 to 2.0 in a solution saturated with salt and of protein precipitation by changes in salt concentration at pH 2.0. Fraction 1 after purification was found not to precipitate in saturated salt solution at pH 6.0–6.2 but did precipitate at pH 5.0; it was completely precipitated at pH 3.5 and 14 g. salt/100ml. solution. Fraction 2 contains at least 2 components, 1 insoluble in distilled water and 1 that is soluble.

M3. A detailed study of the non-protein nitrogen fractions in milk. K. M. SHAHANI AND H. H. SOMMER, Univ. of Wisconsin

Non-protein-nitrogen fractions like urea, ammonia, creatinine, creatine, uric acid and α-amino nitrogen in milk have not been studied as thoroughly as the protein fractions. It is of importance to know what changes take place in the non-protein-nitrogen fractions of milk when the milk is subjected to different plant treatments like pasteurization, homogenization, etc. A detailed investigation with reference to these and also with reference to the effects of feeding has been made.

Total nitrogen, casein, albumin, globulin, proteoses, peptones and non-protein-nitrogen were determined by methods suggested by Menefee, Overman and Tracy with slight modifications. Urea and ammonia were determined according to Perkins on protein-free filtrate by use of urease, and the ammonia was distilled into boric acid, using MgO. Creatine and creatinine were determined colorimetrically, using picric acid and NaOH to develop the color in the protein- and lactose-free filtrate. Creatine first was hydrolysed by acid in an autoclave. Uric acid was determined on the protein- and fat-free filtrate by treating it with NaCN and arsenophosphotungstic acid which resulted in blue color which was compared with a standard. Alpha-amino-nitrogen was determined on milk serum prepared by use of acetic acid and copper acetate. The milk serum was concentrated and Van Slyke determinations were made.

Following data represent the average of 10 composite milk samples as compared with the data of other workers:

	(*mg./100ml.*) (Present results)	(*mg./100ml.*) (From literature)
Total nitrogen	474.07	468.10[a]
Casein *N*	371.96	375.20[a]
Aubumin *N*	37.86	48.90[a]
Globulin *N*	24.12	17.80[a]
Proteoses and peptones	16.25	15.30[a]
Non-protein-nitrogen	23.88	22.97
Urea	8.60	9.33
Ammonia	0.76	0.42[b]
Creatinine	0.46	1.42
Creatine	3.91	2.37
Uric acid	2.26	1.53
Alpha-amino-nitrogen	3.86	4.24
Unaccounted	4.21	4.07

[a] Values reported by Menefee *et al.*
[b] Perkins
Rest by Denis and Minot.

M4. A colorimetric determination of lipase. (A preliminary report.) GEORGE R. GREENBANK AND PHILIP A. WRIGHT, Bureau of Dairy Industry, U.S.D.A.

A rapid method of determining lipase based on the splitting of α-naphthyl esters of the fatty acids is described. The principal substrate is α-naphthyl acetate. Other substrates and their uses are discussed. The color (deep purple) is produced when 2,6-dibromoquinone chloroimine (B.Q.C.) combines with α-naphthol liberated by the lipases. The effect of time, temperature, pH and concentration of the substrate is discussed. It has been found possible to detect the presence of 1 lb. of raw milk in 2,000 lb. of heated milk. The determination may be made in 1 hr. Where the concentration of lipase is low, the reaction time may be increased without appreciable error caused by acid formation or other changes. The use of the method in determining the specificity of lipases is discussed.

M5. Nephelometric determination of fat in non-fat dry milk. BURDET HEINEMANN, E. J. BALDI AND O. B. PARKER, Producers Creamery Co., Springfield, Mo.

The Mojonnier method is the only published method suitable for the determination of fat in non-fat dry milk. The following procedure is the result of attempts to develop a less time-consuming method.

Four g. of NFDM are reconstituted and made to 100 ml. with water. Five ml. are placed in an oil-sample bottle and the following reagents added with mixing: 5 ml. H_2O, 1.5 ml. NH_4OH, 10 ml. alcohol, 25 ml. ethyl and 25 ml. petroleum ethers. Centrifuge 30 sec. and transfer 25 ml. of ether layer to aluminum dish. Evaporate to complete dryness on 135° C. hot plate then float dish on water at 45° C. Add 10 ml. dioxan at 45° C. and swirl 60 sec. Pipette 5 ml. dioxan to 100 ml. pH 2 buffer (Clark and Lubs) containing 0.1% low viscosity carboxymethyl cellulose at 25° C., using a magnetic stirrer at a constant speed. Read 4 min. later in Coleman Universal Model 11 equipped with Nephelometric attachment, setting blank at zero. The curve is obtained by extracting fat from skimmilk, dissolving known quantities in dioxan and adding 5 ml. of the various concentrations (0.05–0.2 mg./ml.) to 100 ml. of buffer under standard conditions.

A complete determination may be made in 20 min. Forty-two comparisons between individual tests by the standard Mojonnier procedure and the above method resulted in a standard deviation of ± 0.09% fat. The range of samples tested was from 0.48 to 1.80% fat.

M6. The action of mineral-ion exchange resins on certain milk constituents. CHARLES W. GEHRKE, Univ. of Missouri, AND EMORY F. ALMY, Ohio State Univ.

When 0.100 *N* solutions of Ca++, Mg++, K+ and Na+ ions as the chlorides are subjected singly to cation exchange using Zeo-Karb-H, the order of adsorption was Ca++ > Mg++ > K+ > Na+ ion. A quantitative relationship existed between the adsorption of the metallic ions from the solution and the hydrogen ions released from the exchanger to the effluent.

The order of adsorption of the metallic ions mentioned above in binary, ternary and quaternary solutions totaling 0.100 *N* was the same as that found for the removal of the cations when they were singly passed through the exchanger.

A variation in the concentration of the constituent ions of the binary solutions did not cause a change in the order of adsorption of the cations studied.

The cations present in simple solutions and in more complex solutions as well (such as milk and whey) which are least adsorbed by the exchanger are found to be removed during the first part of the exchange run, then released later by the regeneration effect of other cations which are preferentially adsorbed.

Ca ions were found to be more effectively adsorbed from a solution containing citric acid than from a solution containing HCl.

The initial removals to the break-through-point for solutions in which the constituent ions have varying concentrations are dependent upon the concentration, type and valence of the component ions of the solution.

M7. Preliminary observations on the electrophoresis study of the proteins in skimmilk. W. L. SLATTER AND Q. VAN WINKLE, Ohio State Univ.

In this study of the components of skimmilk, the electrophoresis apparatus of Tiselius, as modified by Longsworth and Mac Innes, was used. The boundary positions were followed with the Schlieren optical system. Fresh skimmilk was examined at pH 3.1, 4.1, 5.4, 6.6 and 8.5, at ionic strengths of 0.02, 0.10 and 0.50.

At pH 6.6, the descending boundaries indicated that 4 components were present, whereas the ascending boundaries showed only 3 components. The 4th component was indicated by a boundary that moved only slightly and may be wholly or partly an epsilon boundary effect. At this pH, patterns of the descending boundaries were quite different from those of the ascending boundaries, which suggest an interaction between proteins. This interaction between components apparently was at its maximum at pH 6.6.

As the ionic strength was reduced from 0.50 to 0.02, a tendency for the skimmilk to break-up into more components was observed. The 2 components which migrated the most rapidly sometimes were difficult to distinguish at an ionic strength of 0.50, especially at pH 6.6.

When skimmilk was heated to 65, 75 and 85° C. for 30 min. the electrophoretic patterns underwent considerable change. One component disappeared completely from the pattern when the milk was heated to 75 and 85° C. for 30 min.

M8. State of solution of the naturally occurring salts in milk. INDRAPAL S. VERMA AND H. H. SOMMER, Univ. of Wisconsin

The distribution of Ca, Mg, P and citric acid in milk has been studied to determine total amounts and the soluble fractions. The soluble amounts of these constituents were determined by analyzing whey prepared by use of rennet. All 4 constituents were studied on the same sample simultaneously. Only 85% of the total citric acid present in milk exists in soluble form. Analyses of 15 such samples gave the following results (mg./100 ml.):

	Total	Soluble
Ca	132.1	51.8
Mg	10.8	7.9
P	95.9	36.3
Citric acid	154.2	141.6

The influence of pasteurization and cool aging (at 45° for 24 hr.) also was studied on these constituents. There was a decrease in the amount of soluble calcium on pasteurization which tended to come to normal on cool aging and at the end of 24 hr. the amount of soluble calcium in milk was higher than that contained in raw milk. A decrease in the soluble calcium also was accompanied by a simultaneous decrease in the pH and vice versa. These changes in the pH and the phenomenon of retardation of rennet coagulability of milk are explained in terms of precipitation of tribasic Ca and Mg phosphates. The results were supported by the experiments run to study the influence of added Ca and phosphates on the distribution of salts. It finally was confirmed by the behavior of the salts in a solution prepared from pure salts to resemble the composition of salts in milk and at the reaction of normal milk.

M9. Studies on oxidized milk fat. MARK KEENEY AND F. J. DOAN, Pennsylvania State College

Oxidized milk fat was vacuum-distilled to recover the volatile compounds. Major attention

was given to the ether-soluble, neutral fraction, of the distillate. This fraction is a potent flavor concentrate of compounds characteristic of oxidized milk fat. Further separation of the neutral fraction by fractional distillation and reaction with carbonyl reagents indicate that carbonyl compounds are responsible for the predominating odor and flavor of oxidized milk fat. The carbonyl fractions are capable of imparting an oxidized flavor to several million parts of milk.

The identification of these compounds is being attempted by qualitative tests and preparation of derivatives. The hydrazone of a 9-carbon carbonyl compound has been prepared from the neutral fraction and indications are that it is derived from an unsaturated ketone. The hydrazone of a 3-carbon carbonyl compound containing a non-carbonyl oxygen atom also has been prepared. This may be a derivative of lactic aldehyde. Proof of this assumption, however, awaits preparation of an authentic derivative.

The ether-soluble acidic fraction of the milk fat distillate browns readily. The odor, when freshly isolated, is reminiscent of a 6- to 8-carbon acid. In a few days at room temperature it assumes a coconut-like odor. This fraction also reacts with carbonyl reagents. These observations suggest that keto acids are present which slowly dehydrate to lactones.

M10. The enzymatic hydrolysis of lactose in dairy products and its determination. FRANK E. POTTER, Agricultural Research Administration, U.S.D.A.

A colorimetric method has been adapted to the determination of glucose and galactose in the presence of lactose. It is a measurement of the blue color produced by the sugars with $(NH_4)_2MoO_4$ and KH_2PO_4 when heated. The method is based on difference in rate of the color production by the sugars.

The transmittancy at a wavelength of 640 mμ for 1 mg. of lactose in 25 ml. of solution is approximately 99%. An equimolecular mixture of glucose and galactose under similar conditions exhibits 79% transmittancy. When 0.1–5.0 g. of a glucose-galactose mixture was added/100 g. of milk, the average value found for 14 samples was 98.70% of the amount added.

An enzyme capable of hydrolyzing the lactose in whole or concentrated milk products has been supplied by a commercial chemical company. The optimum temperature of enzyme activity is approximately 40° C., but appreciable hydrolysis is obtained at lower temperatures. The enzyme added to condensed skim milk at the rate of 0.5% of the lactose content produced 23% hydrolysis in 5 d. at 4° C. No change in titratable acidity or pH occurred. A comparable hydrolysis can be produced at 30° C. in a few hours.

The hydrolyzed products may be used to produce ice cream of high serum solids content that will not become sandy during storage.

M11. Studies on the water-insoluble acids of butter. F. J. BABEL, Purdue Univ.

Lots of cream testing approximately 35% butterfat were separated from fresh milk. Each lot was divided into 2 portions and held at 55 and 75° F. A sample was taken from each portion daily for 7 d. and at 10 d., neutralized to pH 6.8 with a soda-type neutralizer and churned. The resulting samples of butter were analyzed for water-insoluble acids by the method of Hillig (J. Assoc. Off. Agr. Chem. **30:** 575–582. 1947).

The cream held at 55° F. did not increase in acidity as rapidly as that held at 75° F., nor did it show as rapid development of mold. The % titrable acidity of the samples held at 55 or 75° F. was not materially different after 3 d., although the samples held at 55° F. were always slightly lower until the cream was held for 10 d. The water-insoluble acid content of the butter made from fresh cream was about 240 mg./100 g. fat. The water-insoluble acid content of the butters did not increase materially with an increase in age of the cream at either temperature of holding. There was no significant difference in the water-insoluble acid content of butters made from cream held at 55 or 75° F.

Preliminary data indicate that when cream sours rather rapidly, the lipases are inhibited because of the unfavorable pH. Cream having a clean acid flavor produced butter with a relatively low water-insoluble acid content, while cream having a low acidity but evidence of putrefactive types of microorganisms usually produced butter with a high water-insoluble acid content.

M12. The lactometer as an instrument for determining added water in milk. O. M. YSTGAARD, P. G. HOMEYER AND E. W. BIRD, Iowa State College

Limiting formulae based on changes in serum solids have been proposed for determining added water, c.f. $\frac{S_1 - S_2}{S_1} \times 100$, in which S_1 is the serum solids in the non-watered milk and S_2 those of the sample observed. The legal minimum generally is used as S_1 and obviously invalidates the calculation.

In this study, the Babcock fat test was determined, and the corresponding serum solids from the table presented by Jacobson (J. Dairy Sci., **19:** 170–176. 1936.) was used as S_1. S_2 was determined from the Babcock fat test and the lactometer reading. The lactometric procedure

of Sharp and Hart (J. Dairy Sci., **19:** 683–695. 1936.) was employed; the calculations were made by the following modified Herrington (J. Dairy Sci., 29: 87–89. 1946.) formula:

$$\text{I. } \%\ \text{T.S.} = 1.2537\ \text{F} + \frac{268.0\ (\text{L}+3)}{(\text{L}+1000)} - 0.15$$

Total solids by formula I (T.S._{I}) agreed well with total solids by the Mojonnier method (T.S._{M}). The regression equation relating the two is:

$$\text{II. } \%\ \text{T.S.}_{\text{I}} = 0.9835\ \text{T.S.}_{\text{M}} + 0.2248$$

The correlation coefficienct between the 2 methods is 0.9958.

The percentage added water was calculated by formula III ($\%\ \text{H}_2\text{O}_{\text{III}}$) and from freezing point data ($\%\ \text{H}_2\text{O}_{\text{F.P.}}$).

$$\text{III. } \%\ \text{H}_2\text{O}_{\text{III}} = \frac{\frac{(\text{S}_1 - \text{S}_2)}{\text{S}_1}\ 100 - 0.2}{0.9}$$

The regression equation relating the percentages of water calculated by the 2 methods is:

$$\text{IV. } \%\ \text{H}_2\text{O}_{\text{III}} = 1.019\ \%\ \text{H}_2\text{O}_{\text{F.P.}} + 0.0173$$

The correlation coefficient between the 2 methods is 0.9535.

M13. Rapid salting of brick cheeese.[1] H. J. Buyens and W. V. Price, Univ. of Wisconsin

The brine salting of brick cheese in factory practice usually begins after the curd has drained in hoops for 18 hr. In this experiment the cheese was brine salted after only 6 hr. in metal hoops and the results were compared with normal factory practice.

Salt, moisture and pH measurements were made on a composite sample and on 4 layers beginning with the rind and extending to the center. These tests were made immediately after salting and 4 wk. later at scoring. Tests of composite samples of cheese salted in 22% NaCl brine for 24 hr. showed an average of 1.73% salt in the lots drained for 6 hr. and 1.09% salt in the lots drained 18 hr. This higher trend in salt was most apparent in the outer layers.

The early salting treatment caused a reduction in the amount and firmness of rind formed; this was accompanied by faster "smear" development, smaller moisture loss and higher yield. The quality of the cheese equalled or was slightly better than that of the normal cheese. The early salting treatment makes it possible to hasten the primary changes in curing by more than 24 hr.

[1] Cooperative RMA project with the Bureau of Dairy Industry, U.S.D.A.

M14. Pasteurization of milk for Italian cheese curd.[1] J. C. Marquardt, New York State Dept. of Agriculture and Markets, Albany

The curd commonly is made from milk containing 1, 2, 3 and more than 3% fat. A procedure survey indicated that deviations are not sufficient to change the pH from the range 6.2–6.4 on the fresh curd. Some of the curd is used without further processing. The curd also is shaped after a hot water treatment; the pH range is 5.3–5.5 at this point.

A plant was selected with a flash heater approved by the N. Y. State Dept. of Health. A double check on 48 samples by the State Health Department and the N. Y. City Dept. of Health showed that the milk was pasteurized properly. Curds made from properly-pasteurized milk were superior in flavor to raw-milk curds. There was no problem in molding the cheese made from properly pasteurized milk, although the aging period was increased.

Yields, aging control and a simple procedure for following the aging are being investigated.

[1] Approved for presentation by the New York State Dept. of Agriculture and Markets.

M15. The influence of ultrasonic sound waves on cheese ripening. W. C. Winder, A. M. Swanson and W. V. Price, Univ. of Wisconsin

The application of ultrasonic sound waves to cheddar cheese on a laboratory scale was shown to accelerate the ripening process. Cheese curd either in the whey before dipping or at 1 day of age after removal from the press was treated ultrasonically. Improved cheese consistently was produced when conditions of cheese volume, duration of exposure and wave transmitting media were controlled properly. Measurements of water-soluble nitrogen showed that protein hydrolysis progressed much more rapidly in the ultrasonically-treated cheese than in the untreated cheese. Amino acid assays indicated a more complete protein hydrolysis in the treated cheese; glutamic acid and leucine, which are associated with cheese flavor, were found in increased amounts. Ultrasonic treatment of cheese altered the normal sequence of bacterial development and induced the development of abnormally high populations of streptococci, micrococci and lactobacilli. In simple-system studies the action of ultrasonic sound waves was shown to increase the pH, split lactates and citrates, and produce flavor compounds from organic acids, salts and butterfat.

M16. Observations on an exudate from cheddar cheese. V. L. Zehren and A. M. Swanson, Univ. of Wisconsin

An amber colored exudate occasionally is ob-

served on the surface of cheese during curing. This exuded material is observed more often on cheese cured at higher temperatures. The exudate, when it first appears, is quite fluid, but on exposure to air it becomes viscous and sticky. Rarely is it possible to accumulate any quantity of this material for analysis. A sample of about 3 oz. was obtained from the cheese and shelves of a lot of cheese which had been cured at 60° F.

This is a preliminary report on the analysis of the material exuded from this cheese. The exudate contained 0.6% fat by the Mojonnier method. It contained 65.9% total solids and 34.1% moisture. The ash content was 14.9% of the total solids. The total nitrogen content was 6.5%, of which little was protein nitrogen, as the proteose and peptone nitrogen was 5.7%. The α-amino nitrogen was 2.5%.

M17. The forced-drying of cheddar cheese prior to paraffining. D. M. Irvine and W. V. Price, Univ. of Wisconsin

The drying of cheese before paraffining is a space-, labor- and time-consuming operation. The cheese must be dry before parffin can be applied.

Daisies from the same vat were subjected to normal and forced-drying. Forced-drying consisted of holding the cheese in an air blast at 60–120° F. for periods of from 4–14 hr. The heated air moved over the cheese at the rate of approximately 1,450 ft.^{3}/min.

Cheese held in the air blast at 80° F. for 8 hr. plus 1 hr. at 60° F. lost less weight than the controls. This was the best treatment revealed in this study. Cheese dried at temperatures above 100° F. lost less weight during forced-drying but more during storage than the controls. The quality of the forced-dried cheese was slightly better. Curdiness was retained longer in the force-dried lots. Defects in finish of cheese dried above 100° F. were excessive mold growth, misshapen cheese and a slight exudation of a whey-like fluid.

The 0.25-in. rind layer of the cheese dried at 80° F. averaged 1.8–2.5% more moisture than the controls sampled identically. Weight lost before paraffining averaged 0.915% in 10 lots forced-dried at 80° F., while the comparable normally-dried cheese lost 2.16%.

It seems possible that the time from pressing to paraffining cheddar cheese can be safely reduced to 9 hr.

M18. The free amino acids of foreign type cheese. F. V. Kosikowsky and A. C. Dahlberg, Cornell Univ.

A number of commercial foreign type cheese were analyzed for their free amino acids and other water soluble protein decomposition products, using two dimensional paper partition chromatography. A rough quantitative method utilizing color standards was applied to the analyses of these cheeses. Among the cheese types studied were swiss, roquefort, limburger, leiderkrantz, camembert and Italian.

The free amino acid concentrations were found to be highly variable within cheese types, undoubtedly due to the different ages and past histories of the commercial cheese. Most of the cheese showed about 14–17 free nitrogenous compounds. An example of an extreme case was a commercial muenster cheese which showed within the limits of sensitivity of this method the presence only of small amounts of 2 free amino acids, glutamic acid and aspartic acid.

It was difficult to characterize foreign cheese types from commercial sources by their free amino acid patterns because of the variability of these patterns within types. However, preliminary observations indicate that the average swiss cheese is associated with higher free proline concentrations and the average roquefort cheese with higher free tyrosine concentrations than the other cheeses studied.

M19. The methyl ketones of blue cheese. Stuart Patton, Pennsylvania State College

Blue cheese (roquefort type) has been studied relative to the specific methyl ketones present. The ketones first were separated from the cheese by steam distillation and then, with the exception of acetone, removed from the distillate by ether extraction. Removal of solvent from the extract, followed by distillation of the residue, yielded fractions which were found to be relatively pure 2-pentanone, 2-heptanone and 2-nonanone. These compounds were identified by comparison of data on boiling points, refractive indices and results in certain qualitative tests with those of the known ketones. Identification was confirmed by performance of mixed melting points on derivatives prepared from the unknown compounds and the known ketones. A considerable quantity of material boiling above 200° C. was obtained during the fractional distillation. The column employed did not appear to be effective in fractionating these compounds; however, the results of suitable qualitative tests demonstrated that this material contained traces of additional methyl ketones. Acetone was identified in the extracted steam distillate, the indications being that the ether extraction procedure was ineffectual in removing it therefrom.

The presence of the type ketones identified in these experiments is explainable on the basis of

β-oxidation of milk fat acids. The potent odor and biting taste qualities of these ketones are closely related to the typical flavor of blue cheese. Additional work is in progress concerning the mechanism of the mold action and quantities of ketones produced during the curing of blue cheese.

M20. Tyramine production in cheese and in various bacterial cultures. JOHN A. HUPFER, JR., GEORGE P. SANDERS AND RALPH P. TITTSLER, Bureau of Dairy Industry, U.S.D.A.

Tyramine analyses were run on pairs of cheddar cheese, one made from raw milk and the other from pasteurized milk. The cheese made from raw milk generally contained more tyramine than that made from pasteurized milk and was poorer in quality. The use of poor-quality raw milk resulted in increased tyramine content and poorer-quality cheese.

Pasteurized-milk cheddar cheese was made to compare lactic starter with lactic plus enterococcus. Enterococcus starter greatly increased tyramine production, but did not affect the average quality of the cheese.

Sixty strains of bacteria, representing 28 species and 10 genera, were tested for production of tyramine in milk containing added tyrosine. Tyramine values in μg./ml. of culture were: *Streptococcus durans, S. liquefaciens* and *S. zymogenes,* 228–280; *S. faecalis,* 24–188; *S. thermophilus,* 28; *Microbacterium lacticum,* 48; *Lactobacillus acidophilus,* 0–40; *Leuconostoc citrovorum, Leuc. dextranicum, Leuc. mesenteroides,* 2.5–18; and *L. brevis, L. fermenti* and *L. buchneri,* 24–112. The following gave values of 0–12 μg./ml.: *S. lactis, S. cremoris,* a lactic starter, *Escherichia coli, Aerobacter aerogenes, Bacillus cereus, B. megatherium,* a proteolytic coccus, *Bacterium linens, L. bulgaricus, L. lactis, L. casei, L. plantarum, Propionibacterium shermanii* and *P. freudenreichii.*

M21. The effect of penicillin and streptomycin on swiss cheese starters. R. E. HARGROVE, H. E. WALTER, J. P. MALKAMES, JR., AND K. T. MASKELL, Bureau of Dairy Industry, U.S.D.A.

During swiss cheese investigations, inhibition of starters was noted due to penicillin and streptomycin in the milk supply. A study was undertaken to determine: (a) Percentage of total antibiotics shed in milk by a treated cow; (b) antibiotic sensitivity of cheese starter strains; (c) heat tolerance of antibiotics in milk; (d) inactivation of penicillin by Penase; and (e) the possibility of developing resistant starter strains.

Milk obtained during treatment contained from 26 to 49% of the injected penicillin and from 39 to 58% of the streptomycin. Only 0.04% of the penicillin and no streptomycin remained in the second milking after treatment. The following starter strains showed marked inhibition: *Streptococcus thermophilus* by 0.01 unit penicillin and by 5 μg. streptomycin/ml.; *Lactobacillus bulgaricus* by 0.1 unit penicillin and by 1 μg. streptomycin/ml.; *Propionibacterium shermanii* by 0.1 unit penicillin and by 5 μg. streptomycin/ml. Neither penicillin nor streptomycin was destroyed by pasteurization. Streptomycin was drastically reduced upon autoclaving and steaming, while penicillin was more stable. One ml. of Difco Penase concentrate was required/liter of milk to inactivate 0.5 unit penicillin/ml. *L. bulgaricus* and *S. thermophilus* strains were developed which were resistant to 3 units of penicillin and to 500 μg. of streptomycin/ml., and *P. shermanii* strains to 1 unit of penicillin and to 200 μg. streptomycin/ml.

M22. Observations on a gelatinous curd type of spoilage of cottage cheese. R. B. PARKER, V. N. SMITH AND P. R. ELLIKER, Oregon Agr. Expt. Station

A study was made of a cottage cheese defect in which the curd particles developed a heavy, gelatinous, translucent coating. The defect normally appeared after 2–4 d. storage at 45–50° F. Creaming of the curd appeared to stimulate development of the defect.

Large numbers of an *Alcaligenes* and a *Pseudomonas* species were isolated from both defective curd and the plant water supply. Pure cultures of these organisms produced the same type of defect experimentally. Studies indicated that a pH of 5.0 or lower in the curd mass or hypochlorite treatment of the water supply or both were necessary to prevent development of the defect.

M23. Low cost and small scale methods for concentrating whey for feed. A. H. STEVENS, Bureau of Dairy Industry, U.S.D.A.

Two methods by which the small cheese factory may concentrate whey without the use of vacuum pan were investigated. Whey was evaporated (a) by means of a submerged combustion unit and (b) by spraying it into heated air in a collector similar to those used on a cyclone spray drier.

The submerged combustion unit consisted of auxiliary equipment for supplying air and natural gas to the burner in the correct ratio and at suitable pressure to maintain combustion under the surface of the liquid. Foaming, caused by the turbulent combustion of the air-gas mixture un-

der the surface of the whey, was suppressed by use of a translucent silicone compound. One ft.^{3} of methane gas evaporated 0.69 lb. of water. The whey could be concentrated to about 20% solids, but it had a strongly caramelized color, odor and flavor.

Whey preheated to temperatures above 212° F. was sprayed into a cyclone of heated air during concentration in the cyclone spray evaporator. Results were obtained by use of cones of 2 sizes and with 2 types of sprays during operation at various whey and air temperatures. Whey was continuously concentrated to about 33% solids in this kind of equipment. The physical characteristics of this concentrate compared favorably with those of whey condensed under vacuum.

M24. The utilization of whey in the microbiological synthesis of riboflavin. ABRAHAM LEVITON AND EARLE O. WHITTIER, Bureau of Dairy Industry, U.S.D.A.

Whey is an excellent source of nitrogen and accessory factors for the microbiological synthesis of riboflavin by means of the organism *Ashbya gossypii*. However, lactose is not utilized. By means of mild hydrolytic treatment, which may be incorporated in the sterilization step, yields in excess of 200 mg./l. of whey may be realized on a laboratory scale. The non-heat-coagulable nitrogenous material of whey is the most readily utilized portion. The heat-coagulable portion, on the other hand, contributes but little to riboflavin yield. Maximum efficiency is obtained in dilute whey solutions. A dilution of 1 part treated whey with 1 part water represents a desirable proportion. The use of untreated whey instead of water as a diluent promotes increased yields.

The culture as originally received contained a mixture of cells, some of which were non-productive. By a selective process and over a long period of time, these were eliminated to yield a culture with stable characteristics.

Oxygenation promotes a more rapid synthesis, but does not result in an increase in final yield. In aeration experiments, the use of milk fat and ethylene glycol as foam depressants resulted in poor yields. The use of silicone anti-foaming compounds gave much better results.

M25. Strains of Streptococcus faecalis present in a starter used in the manufacture of cheddar cheese. A. C. DAHLBERG AND F. V. KOSIKOWSKY, Cornell Univ.

In 1946, 2 colonies were selected from azide-penicillin agar plates that proved to be *Streptococcus faecalis* which rapidly produced acid in milk. One starter for use in the manufacture of cheddar cheese was made from them and it has been carried in milk pasteurized at about 200° F. for 1 hr., using a 1% inoculation and 90° F. incubation. The frequency of transfer has been irregular but usually 3 times/week.

In 1949 the question arose as to the purity of the starter. It was plated on tomato juice–yeast extract–tryptone–beef extract–milk agar, and 50 colonies were selected. They all were *S. faecalis* as they were Gram positive streptococci that grew at 10 and 45° C. and in 6.5% salt broth, in penicillin broth (100 units/l.) and in 0.1% methylene blue milk. All cultures reduced and curdled milk and fermented glucose, glycerol and maltose. They decarboxylated tyrosine, hydrolyzed sodium hippurate and showed a green zone on horse blood agar. None of the cultures fermented arabinose, inulin and raffinose.

The predominating strain of bacterium did not ferment mannitol, sorbitol or sucrose and a 1% inoculation reduced and curdled milk in 18 hr. A second strain reduced and curdled milk in 18–48 hr., and it fermented mannitol, sorbitol and sucrose. It should be obvious that, since 2 colonies which curdled milk rapidly were used for the initial starter, both of these strains may have been present. However, a 3rd strain fermented mannitol, sorbitol and sucrose, but it required about 4 d. to curdle milk. This strain could not have been present in the original starter except as an accidental contamination. It is more reasonable to assume that this 3rd strain was a variant of the second, as it is known that *S. faecalis* sometimes loses its ability to ferment lactose.

M26. Bacterial studies of the high-temperature short-time pasteurization of ice cream mix. F. W. BARBER AND H. P. HODES, National Dairy Research Laboratories, Inc., Oakdale, N. Y.

Raw ice cream mix and ice cream mix inoculated with an organism of known heat resistance each were pasteurized in an experimental high-temperature, short-time pasteurizer at 165, 175, 185 and 190° F. for 25 sec. holding time and at 190, 210, 240 and 260° F. for 1.4 sec. holding time. The pasteurization efficiency of the experimental pasteurizer was evaluated by the determination of the per cent destruction of the normal bacterial flora and the heat-resistant test organism and by calculation of the total lethal effect of each pasteurization treatment. High-temperature, short-time pasteurization at 175° F. or higher for 25 sec. holding time and at 190° F. or higher for 1.4 sec. holding time give results that are comparable to the presently accepted pasteurization standard for ice cream mix.

M27. The influence of pH on proliferation of the lactic streptococcus bacteriophage. W. W. Overcast, F. E. Nelson and C. E. Parmelee, Iowa Agr. Expt. Station

Several strains of the lactic streptococci and their homologous bacteriophage strains were studied to determine the influence of pH on phage proliferation. From pH 5.2–9.4 litmus milk was used as the substratum; below pH 5.2, a vegetable juice-peptone broth was used. The pH of the substratum was maintained by frequent electrometric pH determinations and addition of 1*N* NaOH when necessary. The numbers of organisms were determined at intervals by plate counts on TGEM agar. The phages were enumerated by the three-tube limiting dilution technique, using litmus milk fortified with vegetable juice as the medium.

Most strains of phage showed a marked reduction in the rate of increase at pH levels below 5.4; however, some strains were able to increase at pH 4.8. The lag phase at the low pH levels sometimes was prolonged as much as 12–18 hr. When some strains of phage and organism were increasing in milk and the reaction suddenly was lowered to pH 5.2–5.3 by adding lactic acid, phage increase virtually ceased, while acid production continued. When heavy organism inoculations and small numbers of phage particles were used, the rapid lowering of the pH below the critical level for phage proliferation usually prevented occurrence of mass lysis.

With some combinations, phage proliferation virtually was stopped at pH 7.6, even though the organisms increased at a rapid rate. With others, the higher pH prolonged the lag phase but did not prevent later phage increase. Only one phage strain tolerated the high pH as well as the organisms did and this strain showed a definite increase in 24 hr. at pH 9.4.

M28. Changes in bacteriophage and sensitive organism populations in a commercial mixed culture. C. E. Parmelee, F. E. Nelson and W. W. Overcast, Iowa Agr. Expt. Station

Several attempts to isolate bacteriophage from slow vats of milk set with a commercial mixed strain culture had failed when the mixed-strain culture was used as the test culture. The use of single colony isolations as test cultures resulted in the isolation of several phage types.

Transfers from the original culture had been frozen at various times over a 2-yr. period. The sensitivity to 3 phage types of isolates made from first transfers of the frozen cultures varied greatly. Cell-free filtrates made from the first transfer from frozen cultures indicated that the culture carried bacteriophage at different times. At one time 83% of the organisms in the culture were sensitive to one phage type. Incubation of 4 ml. (*ca.* 1×10^9 cells) of this culture and 20 phage particles in 400 ml. of steamed skim milk for 16 hr. at 21° C. resulted in normal coagulation of the milk, complete shift of the organisms to strains not sensitive to the phage added and a titer of 6×10^9 phage particles/ml. in the cell-free whey. Successive transfers of this culture serially diluted the phage until it disappeared. The first transfer of a new culture from the original supplier contained a phage of somewhat similar activity pattern.

M29. A study on psychrophilic bacteria in market milk. F. A. Rogick and L. H. Burgwald, Ohio State Univ.

A study on psychrophiles and their relationship to mesophiles was made on raw and pasteurized milk supplies. Both H.T.S.T. and L.T.L.T. systems were studied over a period of 1 yr.

The experimental data showed that the psychrophiles were not thermoduric and were mostly cocci or non-sporeforming bacilli, inert or acid-producing, facultative rather than true psychrophiles. In every instance the mesophilic counts were higher than the psychrophilic counts in the fresh milk. The mesophilic and psychrophilic counts both showed a definite increase in all samples at the end of 1 wk. of storage in a refrigerator. At this time, however, the psychrophilic count invariably was higher than the mesophilic count.

No psychrophiles ever were found in 4.1 ml. of fresh pasteurized milk taken from the vat (LTLT) or at the cooler (HTST), but at the end of 1 wk. of storage an average of slightly over 50,000/ml. was found. It is thought that some of the mesophiles develop psychrophilic properties. The psychrophilic counts in the fresh, pasteurized bottled milk always were low and attributed to contamination after pasteurization from water used in rinsing equipment, from the equipment itself and, less frequently, from the bottles.

The initial bacterial count of the fresh raw milk was not a true index for determining the further development of psychrophiles.

There was no appreciable difference between the spring, fall and winter mesophilic and psychrophilic counts, but the respective counts were all higher in the summer.

M30. Effects of storage on penicillin in dairy products. W. A. Krienke and E. L. Fouts, Florida Agr. Expt. Station

Pasteurized milk: When added at the rate of 0.25 unit/ml. of milk prior to pasteurization,

penicillin retained its property of completely inhibiting acid production by a lactic starter (*Streptococcus lactis*) through 10 d. of refrigerated storage as was evidenced by a titratable acidity, after 9 hr. of incubation at 95° F., of 0.19% as compared to 0.63% for some of the same milk containing no penicillin, both inoculated with 3% active culture.

Evaporated milk: Although penicillin had been added to whole milk at the rate of 1.0 unit/ml. of milk prior to heat treatment and concentration, there was only a slight effect of penicillin in the evaporated milk immediately after sterilization on acid production of a portion cultured (3%) and incubated at 95° F. for 7 hr. After 9 d. of storage at room temperature its effect on acid production was practically nil.

Condensed whole milk: A portion of the concentrated milk prepared for the evaporated series was placed into refrigerated storage soon after removal from the pan. Another similar portion was mixed with an equal volume of control ("drug-free") and refrigerated also. At both concentrations of penicillin, potency was retained during 13 d. of storage, as evidenced by practically no acid production in these products when cultured and incubated at 95° F. for 8 hr.

Condensed skim milk: At the end of 10 d. of refrigerated storage of the product containing penicillin, acid production, in a portion inoculated at the rate of 3% of lactic culture and incubated at 95° F. for 7 hr., was insignificant.

Non-fat dry-milk-solids: Penicillin was added to skim milk at the rate of 1.0 unit/ml. It was dried in a table model experimental spray drier following the drying of a control sample of skim milk. Periodic examinations, after reconstitution to skim milk, revealed penicillin potency sufficient to greatly inhibit acid production of cultured portions, even when mixed 50–50 with some of the control. There was no detectable loss in potency of penicillin during a storage period of 10 wk. at room temperature.

M31. Organic chelating agents as an aid to dairy detergency. G. A. Claybaugh and J. M. Jensen, Michigan Agr. Expt. Station

Ethylene diamine tetra sodium acetate, used for the improvement of detergents, was studied in the laboratory by an experimental similated washing test in a mechanical washing apparatus described previously by Jensen (J. Dairy Sci., 29: 453–463). The method measured the cleaning efficiency of the detergents in the removal of a prepared raw milk film from glass panes by selected washing procedures. These determinations, obtained by photometric readings, were made by measuring the percentage of light transmitted through the washed panes. Commercially-prepared detergents, as well as experimental mixtures containing various amounts of the chelating agent were tested.

The chelating agent, when used in combination with condensed phosphates and wetting agents, was very effective in aiding the removal of all types of milk films studied. Such combinations easily removed raw milk films prepared by: (a) air drying, (b) oven drying followed by immersion in an alkaline detergent solution and (c) air or oven drying followed by immersion in a chlorine solution. Other combinations of the laboratory-prepared mixtures or commercial detergents such as organic acids, detergent sanitizers and alkaline detergents gave results indicative of incomplete cleaning. When either acid or alkaline detergents were used in hard waters which had been softened by the addition of the chelating agent, excellent detergency resulted. Even with the addition of 10% raw milk to the washing solution, chelating agent-detergent mixtures gave excellent results.

Many combinations of the chelating agent, condensed phosphates and wetting agents were found to be satisfactory detergents for the air-dried, raw-milk films. However, only a few combinations gave good results on the oven-dried, alkaline-detergent film, and the air-dried or oven-dried, chlorine film; these two films were the hardest to remove.

Preliminary data show that combinations of 20–80% chelating agent with a wetting agent and/or condensed phosphates give the highest detergency values. Actual practical applications using these laboratory-prepared mixtures, involving mechanical can washing, substantiate the laboratory detergency values for these compounds.

M32. A comparison of phosphatase tests using different buffers, precipitants and periods of incubation. George P. Sanders and John A. Hupfer, Jr., Bureau of Dairy Industry, U.S.D.A.

The buffers studied included (a) barium borate-hydroxide, this Bureau's method; (b) sodium carbonate-bicarbonate, 21.65 g./l., pH 9.8, Cornell method; and (c) sodium carbonate-bicarbonate, 12 g./l., pH 10.05. Sensitivity was determined in terms of phenol, using 0.1% substrate and using BQC.

With milk and cheese, the sensitivity in the 1-hr. test was greatest with buffer *a* and least with *b;* in the 18-hr. test it was greatest on milk with *a* and on cheese with *c*. With buffer *c*, cloudiness in the color-development stage was eliminated by using potassium oxalate in the trichloroacetic acid precipitant and sodium metabo-

rate color development buffer; optimum pH for color development was found to be 9.3–9.4.

In the 1-hr. test, with buffers *a* and *c*, the optimum pH was about 10.0; with *b* the optimum pH curve was nearly flat with only a slight peak at 9.75. Buffer *c* yielded pH 10 with milk and 9.5 to 9.9 with cheese. It buffers adequately as a single buffer for various dairy products.

Sensitivity differences with these buffers may be due to inhibition by HCO_3^- and $CO_3^=$ ions and/or ionic strength, decreased optimum pH with increased buffer concentration and long incubation, non-optimum substrate concentration in the 18-hr. test and unduly high pH for color development with buffer *b*.

M33. Phosphatase measurements on high temperature vacuum pasteurized churning cream and ice cream mix. G. H. WILSTER AND JUNIUS COVINGTON, Oregon State College

Samples were obtained at the discharge of the 3rd, or high-vacuum, chamber of the vacreator with the normal amount of product being continuously treated. By adjustment of the flow of steam used for heating, the temperature of the cream or ice cream mix in the low-vacuum pasteurizing chamber was varied from 200 or 205 to 170° F. with variations of 5 degrees. Samples were collected first when the highest temperature was used; the temperature then was decreased 5° at a time, allowing 3 min. between each temperature change before obtaining a sample. Fourteen lots of cream and 8 lots of ice cream mix were treated.

To determine if there was any reactivation of phosphatase or if there was any bacterial activity which would result in an increase in phosphatase, all of the samples of vacreated cream, as well as the buttermilk, were tested again at 24-, 48- and 72-hr. intervals. The finished butter was tested again after 7 and 14 d. of storage. All samples were maintained at 40° F. during the holding periods. The "Sanders-Sager" method was used. Negative results were obtained on all samples of heat-treated cream, ice cream mix and butter. Positive results were obtained on the raw products and on the buttermilk. There was no increase in phosphatase after storage for 24, 48 and 72 hr. in any of the samples of vacreated cream, ice cream mix or buttermilk. The butter did not show any increase in phosphatase after storage for 7 and 14 d.

M34. Factors affecting production of proteolytic and coagulating enzyme by Streptococcus liquefaciens. A. T. DUDANI AND F. E. NELSON, Iowa Agr. Expt. Station

The data on heat-inactivation, salting-out and optimum pH indicate that the proteolytic and coagulating enzyme activities of *Streptococcus liquefaciens* probably are just 2 manifestations of the same enzyme. The enzyme is adaptive in character, as all the 9 strains studied failed to produce any detectable amount of the enzyme when grown on Niven and Sherman's simplified amino acid medium (J. Bact., **47**: 335-341. 1944). Two of the strains failed to grow on this medium without added thiamine (5 γ/ml.). In a medium containing 2% vitamin test casein, 6 vitamins (as used in the Niven and Sherman medium), 1% KH_2PO_4 and 0.2% lactose, the various strains produced different amounts of the enzyme as determined by the milk coagulation test and the freeing of tyrosine and tryptophan from casein.

In case of only one of the nine strains, addition of B_{12} (0.0004 γ/ml.) to the casein medium resulted in a 3-fold increase in enzyme production. Substitution of B_{12} with cobalt failed to produce the same effect.

In milk, two of the strains showed maximum enzyme activity after 18 hr. incubation at 35° C. Incubation of cultures for longer periods resulted in gradual decrease and ultimate inactivation of the enzyme. This is believed to be due to longer exposure to the low pH of old milk cultures.

M35. Taking representative milk samples from weigh tanks. J. C. MARQUARDT, N. Y. State Dept. Agr. and Markets, Albany

An attempt has been made to identify the characteristics of weigh tanks which offer the best possibilities for representative sampling of milk for fat determinations. In a satisfactory tank, results on successive samples taken from the regular sampling port both immediately after dumping the milk and after vigorous agitation show no distinct trend to vary beyond the normal inherent variation of results by the Babcock method.

After correlating the capacity and the dimensions of 360 tanks with fat tests on milk from many of them, limited predictions are believed possible concerning the suitability of certain installations. Guided by this predictability, observations were made on 10 standard and on 5 special tanks. Determinations showed slightly better agreement in special tanks than in standard tanks. Establishing specifications for standard tanks may be unnecessary. New installations should be checked and old ones should be rechecked at regular intervals. Only experienced personnel should make conformance determinations.

A dye procedure has been used for determining

conformance characteristics in plants where objective checking as described above is difficult.

M36. Determination of quaternary ammonium compounds in milk, and in detergent sanitizer and buffered quaternary solutions. D. D. MILLER AND P. R. ELLIKER, Oregon Agr. Expt. Station

A modified eosin-indicator procedure has been developed for determination of concentration of added quaternary ammonium compounds (QAC) in milk and certain other foods. The method also has been adapted to determination of QAC in detergent sanitizer and in buffered QAC solutions. The essential steps in the procedure include: (a) Extraction and precipitation of QAC in a tetrachloroethane-acetone-eosin indicator solution; (b) Removal of interfering factors by washing the extraction solvent with successive portions of distilled water; (c) Titration of QAC extracted with standard anionic solution.

Recovery in the solvent fraction consistently has approximated 80% of the original QAC present in the sample. The remaining 20% can be recovered by repeating the extractions on the distilled wash water fractions. Trials included addition of 11 different QAC- and 3 QAC-containing detergent sanitizers to milk and tests to recover added QAC. Total recoveries within plus or minus 2 ppm. were obtained on concentrations ranging from 5–100 ppm. QAC in milk. Some QAC preparations could be detected in milk in concentrations lower than 5 ppm.

M37. Ion exchange as a means of improving the keeping quality of frozen homogenized milk. H. S. HALLER AND R. W. BELL, Bureau of Dairy Industry, U.S.D.A.

Ion exchange treatment of milk, in most instances, deferred the formation of an oxidized flavor during frozen storage. However, some exchangers imparted a slight salty or other foreign flavor when the treatment was too vigorous or prolonged. The physical stability of the frozen milk varied with different types of treatment. Removal of about a 4th of the Ca and lesser amounts of the other cations with no removal of the citrates, phosphates or chlorides resulted in definite improvement in physical stability. However, removal of approximately these same amounts of cations together with half of the citrates, phosphates and chlorides greatly reduced the stability. Removal of about 20% of the citrates and a small amount of Ca and other cations resulted in a slight decrease in stability.

The most satisfactory treatment involved the use of an organic cation exchanger regenerated to a 99: 1 sodium-hydrogen ratio. This treatment, by removal of as little as 10–15% of the Ca and lesser amounts of the other cations, with no removal of the anions, produced a normal flavored milk with improved physical stability and deferment of an oxidized flavor.

M38. Observations on the effect of additions of heat thickened protein to fluid milk on the creaming phenomenon. A. C. SMITH AND F. J. DOAN, Pennsylvania State College

Some eastern milk dealers have been increasing the cream volume of their bottled milk by the illegal practice of standardizing high test milk by addition of reconstituted superheated condensed skim milk. The expansion of the cream volume apparently depends on the entanglement of protein flocs in the rising fat globule aggregates and its degree is influenced by the amount of superheating of the adulterant and by the proportion added. Maximum increases in cream volume result when the ratio of fat to superheated protein ranges between 2.85 and 4.0.

Positive detection of this type of adulteration is not easy. Adulterated milk exhibits normal appearance, flavor, alcohol number, pH, freezing point, surface tension and tyrosine values. The nitroprusside test for —SH groups, the Evanson test for "bound" lactose and the test for furfural on the steam distillates are negative. Microscopic observation (with and without staining techniques) of the mixed milk and of the cream layers reveals no distinguishing criteria. The curd content of butter churned from the cream layers is normal.

Adulterated milk shows more sediment on centrifuging and a higher ratio of casein to albumin and globulin; the cream layer exhibits subnormal fat-to-casein and fat-to-s.-n.-f. ratios; and the under layer is higher than normal in viscosity by the Ostwald pipette. The fat-to-s.-n.-f. ratio of the cream layer appears to be the most convenient of the significant criteria for detection of the adulteration.

M39. The effectiveness of some antifoaming agents in the condensing of skimmed milk and whey. J. ROBERT BRUNNER, Michigan Agr. Expt. Station

Milk, cream and butter as well as selected surface active compounds were added to skimmed milk and whey to control excessive foaming during the condensing operation. Fresh cream and sweet cream butter, used to provide a milk fat concentration not exceeding 2% in the skimmed milk and whey, were moderately effective as foam depressants. However, the addition of much smaller amounts of slightly rancid milk or cream were extremely effective.

Surface active compounds used were divided into 2 groups according to their respective solubility in water. The water-insoluble materials, listed in the order of their effectiveness as antifoaming agents, were silicone materials, mono- and diglyceryl esters of high molecular weight fatty acids, sorbitan monolaurate and octyl alcohol. The water-soluble, surface-active materials studied were butyric acid, a cationic quaternary ammonium chloride, an anionic alkyl aryl sulfonate and a nonionic polyoxyethylene sorbitan monolaurate. The foam-inhibiting property of butyric acid and the cationic quaternary was pronounced, but in both instances the collapse of the foam film was accompanied by a partial coagulation of the milk proteins. The addition of either polyoxyethylene sorbitan monolaurate or the alkyl aryl sulfonate to skimmed milk and whey enhanced the formation of copious amounts of a very stable foam.

Since the water-insoluble, surface-active compounds were most effective as antifoaming agents during the condensing of skimmed milk and whey, the foam depressing property of rancid milk and cream possibly could be attributed to the presence of water-insoluble, surface-active materials formed as a result of the partial hydrolysis of milk fat. With the exception of octyl alcohol, none of the antifoamants, when used in effective concentrations, imparted an off-flavor to the condensed products.

M40. The antioxidant properties of nordihydroguaiaretic acid in cream pasteurized at various temperatures. B. T. Karnani, Dionisios A. Theokas and Vladimir N. Krukovsky, Cornell Univ.

The addition of NDGA antioxidant to portions of cream prior to their pasteurization at 143, 150, 160 and 170° F. for 30 min. (0.005% of the bulk fat) and which were either depleted of the total vitamin C content or to which vitamin C (20 mg./l.) and Cu (0.4 mg./l.) were added alone or together, resulted not only in the increase in apparent tocopherol values of the fat, but also in the stabilization of cream and fat against deterioration (as determined by the re-emulsification test) for at least 180 d. at sub-zero temperatures and for an additional 20 d. at 0–1° C.

The pasteurization of cream at 143 and 150° F. without NDGA antioxidant resulted in the prevention of oxidized flavors in both cream depleted of the vitamin C content and cream containing added vitamin C for 180 d. at subzero temperatures and an additional 20 d. at 0–1° C. However, the fat became unstable at the end of 75 d. at sub-zero temperatures, and, in the case of creams pasteurized at 143° F., at the end of 10 and 20 d. at 0–1° C. after approximately 60 d. at sub-zero temperatures. The creams containing Cu or added Cu and vitamin C developed oxidized flavors at the end of 75 d. at sub-zero temperatures, and the fat became unstable at the end of 15 d. at sub-zero temperatures.

The pasteurization of cream at 160 and 170° F. without NDGA antioxidant prevented the oxidized flavors in cream depleted of the vitamin C content, and cream containing added vitamin C for 180 d. at sub-zero temperatures and the additional 20 d. at 0–1° C. During this time only the fat from cream pasteurized at 160° F. and containing added vitamin C became unstable at the end of 75 d. at sub-zero temperatures. The same cream, but containing Cu or Cu and vitamin C developed oxidized flavors at the end of 45 d. at sub-zero temperatures, and the fat became unstable at the end of 15 d. at sub-zero temperatures. The creams pasteurized at 170° F. and containing Cu alone and together with vitamin C developed oxidized flavors, and the fat became unstable at the end of approximately 10 d. at 0–1° C. following 180 d. at sub-zero temperatures.

M41. Stability of evaporated milk as influenced by various conditions of homogenization. R. B. Maxcy and H. H. Sommer, Univ. of Wisconsin

Applying the normal process of manufacturing evaporated milk, the temperature of homogenization had little, if any, effect on the heat stability of the finished product. On the other hand, if the milk had been homogenized prior to forewarming and condensing, the temperature of homogenization exerted a profound influence on the final stability. There was a marked increase in stability as the temperature of homogenization was increased from 100° F. up to near 180° F.

When the milk had been homogenized at 160° F. or above as unconcentrated milk, the stability could be improved 5–6 min. at 242° F. by rehomogenizing the product at 130° F. after it had been concentrated.

When cream containing 20% butterfat was homogenized at 136° F., then mixed with forewarmed and concentrated skim milk to obtain a product with the composition of evaporated milk, the product was fairly stable, but the stability could be improved 5–6 min. at 242° F. by rehomogenization at 130° F.

Evaporated milk prepared in the normal manner was homogenized as many is 7 times at 130–135° F. without detrimental effect to the stability beyond that of the first homogenization.

M42. Separation of fat and protein in sterilized milks during storage. B. H. Webb, E. F. Deysher, C. F. Hufnagel and F. E. Potter, Bureau of Dairy Industry, U.S.D.A.

Sterilized milks of different concentrations, including evaporated milk, exhibit various degrees of fat and protein separation during storage. Excessive separation is objectionable and it does not occur in properly processed evaporated milks held under normal storage conditions. Poor homogenization, inadequate heating during sterilization, low viscosity and high storage temperatures accelerate phase separation. Thus far, it has not been possible to improve the color and flavor of these milks by rapid sterilization because of the destabilizing influence of this kind of sterilization on the fat and protein dispersion. Two high-temperature short-time methods may be used to sterilize plain or evaporated milk: (a) sterilize the milk in cans with violent agitation at about 260° F. for 3 min. and (b) sterilize the milk with steam jets or in a tubular heater at about 285° F. for 15 sec., then package it aseptically. Milks so sterilized have a very thin body, which allows rapid separation of fat and protein, and a storage life of only a few months. When protein separation in sterilized milk does occur, it appears first as a precipitate, but in later stages, the high localized concentration of protein seems to be conducive to gel formation. If the protein settles, the gel appears on the bottom of the can. If the protein is adsorbed on the fat phase, which rises, redispersion of the gel-like fat layer becomes almost impossible. Phase separation proceeds more rapidly in sterilized skim milk than in whole milk. The fat and protein, when bound together by adsorption, partially stabilize each other. If all of the fat and protein of milk could be combined by adsorption and dispersed as small units, a balance might be obtained which would retard separation regardless of the viscosity of the milk.

M43. The use of concentrated essence for improving the flavor of strawberry and peach ice cream. C. C. Flora, L. L. Davis and C. W. Holdaway, Virginia Agr. Expt. Station

In the Horticultural Dept. of the Virginia Agr. Expt. Station, concentrated strawberry and peach essences were processed by a specially designed fruit-essence recovery process for supplying concentrated volatile flavors. These essences were used with frozen and canned strawberries and peaches in making strawberry and peach ice cream.

When concentrated strawberry and peach essences prepared by the above process were added to fruits for use as an ice cream flavor, they fortified and greatly improved the natural flavor of this fruit. This fortified flavor carried through to the finished ice cream. The improvement in flavor seemed to be somewhat better when essences were used with the canned strawberries and peaches rather than with the frozen strawberries and peaches. Concentration of 20 ml. of approximately 100-fold essence/5 gal. of mix rated superior to that of higher or lower concentration.

M44. Some relationships of the oxidation-reduction systems of milk to the keeping quality of the dry product. H. A. Harland, S. T. Coulter and R. Jenness, Univ. of Minnesota

Twenty lots of dry whole milk were prepared from fresh milks that had been subjected to a variety of preheating conditions ranging from 85° C. for 25 sec. to 96° C. for 30 min. These milks were examined following each step in the processing for their oxidation-reduction potentials and assayed for substances that reduce thiamin disulfide, acid ferricyanide, O-iodosobenzoate and 2,6-dichlorophenolindophenol. In addition to these tests, flavor scores and fat peroxides were determined on the dry milks initially and following biweekly intervals of storage in air at 37° C.

Treatment of the fluid milk at 96° C. for 75 sec. produced dry whole milk with the best keeping quality as judged organoleptically and by means of fat peroxide determinations on the extracted fat. Moreover, this heat treatment produced the maximum amount of thiamin disulfide-reducing substances (TDRS) in the milk. While there may be no direct proof for the antioxygenic properties of TDRS in milk, a close relationship exists between the amount of TDRS present in dry whole milk and the storage life of this product in air.

The iodosobenzoate-reducing substances in milk decrease with increasing time of heat treatment of fluid milk in air but do not show further decrease during 10-wk. storage of dry whole milk in air at 37° C. Furthermore, no direct relationship is evident between the quantity of iodosobenzoate-reducing substances and fat deterioration during storage of dry whole milk.

Since the oxidation-reduction potential of milk depends largely upon the O_2 tension and the ascorbic acid content, determinations of the relatively small changes in E_h that occur during processing and storage of dry whole milk are of limited value for studies of the relationships of processing changes in milk to the keeping quality of the dry product.

M45. A disc method of filtration for roller process non-fat dry milk solids. D. R. Strobel and C. J. Babcock, U. S. D. A.

The filtration method for determining sediment content generally is more accurate, more uniform and faster than the tumbler method. However, the insoluble solids in reconstituted roller nonfat

dry milk solids prevent sediment determination by filtration through the standard lintine disc. A filtrable solution was produced by a pepsin-hydrochloric acid solution (10 g. pepsin in 500 ml. water plus 25 ml. HCl, diluted to 1,000 ml.) prepared as follows: Mix 25 g. of the milk powder in 125 ml. of the solution, heat to 40° C., hold 40 min., boil 10 min., then filter. The sediment content determined by this method equaled or exceeded that determined by the tumbler method and gave a truer and more complete picture of the sediment content of the samples than that shown by the tumbler method. The discs clearly showed scorched particles, burnt particles and other sediment.

Results will be presented of tests on 200–300 samples, comparing the best established procedure with the tumbler method.

M46. Comparison of the yield of non-fat dry milk solids when using three types of spray drying equipment. BEN M. ZAKARIASEN AND GUNNAR E. NELSON, Land O'Lakes Creameries, Inc., Minneapolis, Minn.

In recent months many inquiries have been received as to which type of spray drying equipment will produce the best product at the lowest cost per pound. An important factor in determining which type of drying equipment to purchase, is the yield of non-fat dry milk solids per pound of solids in the original liquid skimmilk.

In this study, an attempt was made in a practical way to compare the yield using 3 types of commercial spray drying equipment. One type was inferior from the standpoint of yield of non-fat dry milk solids per pound of solids in the original skimmilk.

M47. Effect of heating on the diffusion of calcium, magnesium, phosphorous and citric acid. T. D. HARMAN AND W. L. SLATTER, Ohio State Univ.

The effect of heating skim milk on the rate of diffusion of Ca, Mg, P and citric acid was studied using an adaptation of the Hanke and Koessler method of dialysis. Fresh skim milk was dialyzed against an isotonic solution of lactose and NaCl, using a cellophane membrane calibrated with a standard solution of these ions. Two series of heat treatment were used. In the 1st, skim milk was heated to 62, 77 and 100° C. for 30 min., and in the 2nd series, the skim milk was heated to 100° F. for 30, 60 and 90 min. In each case raw milk served as a control.

In all cases the rate of diffusion of the Ca, P and Mg was decreased markedly, whereas the citric acid apparently was unaffected. The decrease in the rates of diffusion of the Ca, Mg and P was essentially the same when the skim milk was heated to the various temperatures for 30 min. The extension of the heating period to 60 and 90 min. resulted in further decreases in the rates of diffusion which were not directly proportionate to the increased time of heat treatment.

In the case of Ca, Mg and citric acid, the diffusion resembled a first order reaction, but the P diffusion did not.

M48. The operation of a spray drier at high temperature and under pressure. V. H. TOWNLEY AND S. T. COULTER, Univ. of Minnesota

A pilot model of the Minnesota spray drier, equipped with a gas burner to supplement the steam heaters, was used to study the effects of varying the inlet air temperature and the absolute air pressure on (a) the drying rates, (b) the temperature of the powder during drying, (c) the moisture content of the powder produced and (d) the solubility index of the powder.

Both high-temperature inlet air and high absolute air pressure in the drier would increase the capacity and the former would increase the thermal efficiency of the drier. The time necessary to dry the powder to 5% moisture content apparently was dependent on the nozzle characteristics and on the water vapor pressure of the exit air. Neither the inlet air temperature nor the air pressure had any apparent effect on the temperature of the drying particles nor upon the solubility index of the powder. To maintain a constant outlet air temperature, it was necessary to increase the milk flow 300% with increase in inlet air temperature from 270 to 410° F. Increase in air pressure necessitated an increase in milk flow in direct proportion to the density of the air in order to maintain constant outlet air temperature. The use of these high milk flow rates caused an increase of 1.5% in the moisture content of the powder, presumably due to increase in a vapor pressure of the outlet air. This increase in moisture content would not be a bar to the use of these methods since a redrier could be used to remove the excess moisture.

M49. The changes produced in the ultrafilterable calcium, phosphorus, and nitrogen components of skim milk during processing in a Mallory heat exchanger. E. A. BERNARDONI AND S. L. TUCKEY, Univ of Illinois

Six lots of fresh skim milk, one lot of skim milk plus 0.06% $CaCl_2 \cdot 2H_2O$, and 2 lots of sweet whey each were heated in a Mallorizer to the following temperatures: 325, 300, 250 and 200° F. The samples were filtered through a no. 2F porcelain Pasteur-Chamberlain ultrafilter in a Briggs chamber under 90 lb. pressure.

The ultrafiltrate from each lot was analyzed for: total N, amino N, Ca and P. The average total N content of the filtrate from the skim milk samples heated to 200, 250. 300 and 325° F. was 92.4, 87.4, 93.6 and 104.6%, respectively, of the raw skim milk filtrate. However, the whey samples showed a continuous decrease in total N with increased temperatures. The amino N content in the filtrate from the skim milk samples increased as the temperature was raised above 250° F. The Ca and P content in the ultrafiltrates decreased as the temperature increased. At 325° F., the average decrease was 15.7 and 10.4%, respectively, when compared with the filtrate from the raw sample. At 325° F. for the whey sample this decrease amounted to 30.3% for the Ca and 13.7% for the P.

M50. Effect of high temperature short time heat treatments of milk on the denaturation of albumin and globulin. J. H. HETRICK, Dean Milk Co., Rockford, Ill. AND P. H. TRACY, Univ. of Illinois

Milk was heated, held and cooled continuously by means of a Mallory heat exchange unit. Calculated times of 4.2 and 1.6 sec., respectively, were required to heat the milk to and cool the milk from the desired holding temperatures.

Total N, non-casein N and non-heat denaturable N were determined in duplicate on the experimental samples. The % of the total amounts of albumin and globulin denatured were calculated both from the increases in casein N and from the decreases in albumin and globulin N. No attempt was made to determine either albumin N or globulin N separately so that the data refer to the denaturation of a fraction which includes both albumin and globulin. The percent of the total albumin and globulin denatured by a standard heat treatment was reproduced satisfactorily by the methods employed when different lots of milk were used. These values were not affected by variation in the total N content.

When the heat treatment employed was limited to minimum time-temperature conditions for inactivation of the phosphatase, no denaturation of albumin and globulin occurred. The extent of denaturation of albumin and globulin with time at a constant temperature of 170° F. followed closely the first order law, but at 230° F. this was not the case. Heat treatments sufficient to cause the first noticeable cooked flavor resulted in the denaturation of about 58% of the albumin and globulin. Data are given showing amounts of albumin and globulin denatured by heat treatment in the temperature range from 180 to 322° F. with calculated holding times at top temperature of 0.03, 26 and 64 sec. Data also are given which show the importance of rate of heating to a given temperature on the amounts of albumin and globulin denatured.

M51. Lactose degradation in heated milk. STUART PATTON, Pennsylvania State College

Earlier work demonstrated that furfuryl alcohol, maltol and hydroxymethylfurfural are formed from lactose in heated milk. Present studies are concerned with the conditions which favor the generation of these compounds. Condensed skim milk (30% total solids) and a number of simplified systems, employing lactose in combination with other materials, were used in these experiments. The compounds were isolated from the heated samples by a continuous ether extraction technic.

Milk samples heated 127° C. for 2.5 hr. at pH 6.5, formed very little hydroxymethylfurfural but significant quantities of maltol and furfuryl alcohol. The reverse was observed to be true with milk samples adjusted to pH 4.8 prior to heating. Results with the heated lactose systems indicated that weakly basic conditions favor the production of furfuryl alcohol. This compound was isolated from systems of lactose combined with casein, lysine, or $NaHCO_3$, and in smaller amounts from a heated solution of lactose in phosphate buffer (pH 6.6). In conjunction with the experiments on furfuryl alcohol, it was observed that amino acids or casein are somewhat specifically required for the heat generation of maltol from lactose. Maltol was obtained from heated systems of lactose combined with casein, lysine and, to a lesser extent, glycine. Heating lactose in the presence of dilute $NaHCO_3$, NH_4OH or NaOH produced no maltol.

The heat degradations of glucose and galactose were studied under a wide variety of conditions but in no instance was furfuryl alcohol or maltol observed to be formed from these sugars.

EXTENSION SECTION

E1. Master package for integrated county meetings on dairy farm management. STANLEY N. GAUNT, Massachusetts State College

Our Extension Dairy Committee each year reviews the problems of the industry and develops an educational program to meet them. To meet this year's major problem of adjusting farm operations to smaller net incomes because of lower milk prices and high production costs, a "master package for integrated county meetings on dairy farm management" was developed. This package consisted of 4 meetings with the first one presenting the situation from a survey of production costs and how dairymen can analyze their

problems. The 3 following meetings covered how to increase the net farm income.

Development of this package included: (a) the organization of subject matter and its assignment to 7 specialists; (b) preparation of supporting and supplementing subject matter; (c) planning and constructing effective visual aids; (d) a preview before county agents of each specialist's talk; and (e) preparation of suggested circular letters, news stories and highspots of each meeting.

The preview convinced county agents that these packaged meetings were different and better. The result was more dairy meetings, particularly in certain counties, more dairymen attending and, according to most people, the best organized and presented series of dairy meetings held in Massachusetts in recent years.

E6. Effective methods in promoting the junior dairy project. GLEN W. VERGERONT, Univ. of Wisconsin

Extension dairymen working in an advisory capacity with state 4-H Club departments and county extension agents have suggested several plans which have been helpful in promoting the junior dairy project. State breed associations, service clubs and active groups of dairymen have helped boys and girls to locate, finance, select and draw suitable calves on various practical plans. Judging schools, judging contests, tours, fitting and showing demonstrations, heifer sales, dairy demonstrations and twilight meetings have helped advance the work.

Suggested quarterly and yearly plans of work are used in many states to get an orderly approach to the teaching of efficient dairy production fundamentals as the activities enumerated above.

District project meetings for county extension agents and leaders and some county leadership meetings have been assisted by extension specialists.

Visual aids have been used at meetings and handicraft projects and practices have been suggested in the order of their popularity as follows: rope halters, blankets, hay racks, techniques in foot trimming, feed boxes, calf stalls, false bottom calf pens, tools, electric dehorning and milk stools.

E7. How the American Dairy Science Association and the United States Department of Agriculture can aid in the development of 4-H Dairy Club plans and programs. E. W. ATTON, U.S. D.A., Washington

In Dairy 4-H Club work there can be a happy combination of emphasis which results in a 4-fold development of the boy or girl and the economic progress of the farm and community. Dairy members remain in Club work longer than those who enroll in most other projects. Dairy and livestock projects are well adapted to the development of family partnerships with the parents. 4-H education in meal planning helps build strong bodies and the kind of teeth that encourage youth to smile and enjoy life.

The American Dairy Science Association and the Cooperative Extension Services of the United States Department of Agriculture and the States should develop some joint projects of research and study. A few problems or questions that might be tackled for a start are:

1. What are the "success factors" of our present 4-H Dairy programs?

2. Is it educationally sound to expect youth of 10 and young adults of 20 to participate on the same basis in the same program and the same club?

3. Is individual competition of one member vs. other members the best and only effective stimulation toward greater effort? Is it possible that some of the group competition used so successfully in the Young Farmers Clubs of England, Scotland, Wales and Ulster might also prove to be successful here in the United States for holding the interest of older members?

4. Many new educational methods and techniques have been devised in the past 30 yr. Have we kept pace?

5. What are the values and methods of bringing more youth participation into the planning and action phases of 4-H Dairy programs?

E11. Hyperkeratosis (X-disease) of cattle. P. OLAFSON, Cornell Univ.

Hyperkeratosis is a chronic disease of cattle of unknown cause. Over 30 scattered outbreaks of the disease have been diagnosed in New York State since the original recognition of the condition in 1941. The disease has appeared in more than 30 states.

This is an insidious chronic disease which may have been in progress for weeks before the owner suddenly realizes that serious trouble is present. The early manifestations are interpreted as lousiness, mange or other skin diseases. Antiparasitic treatments fail and the disease continues to progress. The chief symptoms are watery discharge from the eyes, salivation, loss of weight, depression and a progressive thickening and wrinkling of the skin. The skin changes start over the withers, sides of the necks and on the cheeks. Severe cases may lose most of the hair and the upper two-thirds of the body may be covered by altered skin. Wart-like proliferations

may be present in the mouth. Examination of the internal organs will reveal marked changes in the liver, kidneys and pancreas. There is proliferation of the bile and pancreatic ducts, as well as cystic dilatation of kidney tubules. Affected animals have little resistance to pus-producing bacteria which results in frequent development of mouth, skin and liver abscesses. Young animals ranging from 4 to 24 mo. of age are affected most frequently.

Experiments are being conducted to determine whether this is an infectious, toxic or metabolic disease. The problem has proven to be a difficult one and so far no one has succeeded in producing the disease experimentally.

E12. Method of conducting an educational program for area brucellosis control. E. C. SCHEIDENHELM, Rutgers University

The New Jersey dairy extension program has a sub-project entitled, "Farm sanitation and animal health". This, in the main, is an educational program complementing the regulatory program of the State Bureau of Animal Industry. Through a long-time program an attempt is being made to have the entire state become a modified accredited area for brucellosis.

Area work for control of brucellosis has been set up on a township test basis. Counties with low cow population are used to start with and eventually all counties will be included. Steps in developing a township program are: (a) Convince agricultural agent program is necessary. (b) Meet with county board of agriculture and secure their approval. (c) Educational meeting to get a majority vote of farmers in the selected township. All testing is done on a voluntary basis. Each dairyman uses results to select the brucellosis control program best adapted to his particular problem.

E14. Bacteria in the cow's udder associated with mastitis. J. J. REID, Pennsylvania State College

An investigation of mastitis has been conducted during the past 9 yr. It has included studies of the organisms associated with udder trouble and the relation of certain management practices to the incidence of the disease. More than 40 herds have been included in the investigation representing institutional and farm herds throughout Pennsylvania. The relative incidence and importance of *Streptococcus agalactiae* has been overemphasized in the literature. Data obtained indicate that this organism is seldom, if indeed ever, the agent found associated with an initial case of mastitis but rather the organism which may eventually become of importance following a period in which udder resistance falls to an extremely low level.

Organisms found associated with initial cases of udder trouble include pyogenic *Micrococcus* species, coliforms and, particularly in the case of older animals, *Streptococcus* species other than *agalactiae*. *Pseudomonas aeruginosa* has been found with some of these organisms in certain herds. *Corynebacterium* species do not appear to be of importance in mastitis developing during lactation. In many cases it has been found impossible to isolate bacteria from milk obtained at the onset of trouble.

Data further indicate that the cause of mastitis is related to management practices or injuries which lower the resistance of the udder. The organism eventually found associated with the trouble is purely adventitious in nature.

DETERMINATION OF MILK MINERALS BY FLAME PHOTOMETRY[1]

R. J. KEIRS AND S. J. SPECK[2]

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This article is an outgrowth of the authors' need for a method for the determination of the minerals in milk during a milk composition study involving the determination of some 15 to 20 samples per month over a 3-yr. period. The authors sought a method not as laborious nor as time consuming as the standard chemical methods but still having an accuracy within 1 to 2 per cent of the total amount present. The investigation was undertaken to establish a flame photometric method for calcium, sodium, potassium and magnesium using a Beckman D.U. spectrophotometer with flame attachment (17). The authors succeeded in the cases of the first three elements, but failed to establish a method for magnesium, principally because of the low concentration of magnesium in milk. Milk averages 0.012 per cent magnesium (10), while the upper limit of concentration of magnesium determinable to 1 per cent of the amount present by the Beckman flame photometer is 0.2 per cent (17).

Flame photometry is a relatively new adjunct to analytical chemistry. Several papers (2, 3, 4, 9, 16, 19, 20) have described flame photometric methods, techniques and improvements for samples including biological materials using instruments other than the Beckman. Brown *et al.* (6) describe a method for calcium and magnesium in leaves using a Beckman instrument, but no method has been published specifically for minerals in milk.

Several techniques were studied before deciding upon the method described. The absolute method of determining an unknown concentration by interpolating flame intensity readings on a preplotted working curve prepared from known standard solutions first was tried with little success regarding accuracy and reproducibility. The internal standard technique proposed by Gerlach (7) for spectrographic analysis and applied to flame photometry by Berry *et al.* (3) also was investigated. While this latter technique increased precision over the absolute method, results still were not satisfactory. A modification of the absolute method similar to that employed by Gilbert (8), but simpler in that background readings and a calcium background curve are eliminated, was studied and employed with success. In this method the concentration of the unknown is determined by interpolating the relative intensity of the unknown between the in-

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tensities of two standards, one of slightly lower concentration and the other of slightly higher concentration than the unknown. The three readings are taken within seconds of each other to reduce chance for variation in the flame or instrument during the readings.

EXPERIMENTAL

A Beckman D.U. spectrophotometer (17) with flame attachment was used throughout this study. Although numerical results were not tabulated, it was observed visually that with illuminating gas a steadier response was obtained on the galvanometer than when propane was used. For this reason, illuminating gas was used throughout. The gas pressure was maintained at 3.5 cm. of *n*-butyl alcohol (manometer fluid). This pressure gave a clear cone-shaped flame at oxygen pressures used for each of the elements without danger of backfiring. The optimum oxygen pressure was determined for each element by plotting the spectral intensity of each element as oxygen pressure was varied.

It was necessary to preheat the spray chamber at least 10 min. before taking the first of a series of readings to allow the chamber to reach an equilibrium temperature. The solvent used was atomized into the spray chamber during this preliminary heating; otherwise the temperature would rise above the operating temperature and the first few readings taken would be erratic until the equilibrium temperature was established again. Alcoholic solutions (20 per cent by volume) gave a steadier flame and were used in preference to aqueous solutions.

Correction for flame background was not made. Preliminary investigation showed that, for a given slit width and with the preparation of standards of the approximate composition as the unknowns, the flame background at any particular wavelength remained constant and was the same for a given ion whether in standard or unknown solution.

The effect of each of the ions in solution upon the intensities of calcium, potassium and sodium was studied. This was done by preparing, as a standard of reference, a solution with the following ion concentrations: calcium, 12 p.p.m.; potassium, 12 p.p.m.; sodium, 4 p.p.m.; phosphorus, 12 p.p.m.; magnesium, 1 p.p.m.; hydrochloric acid, 0.2 ml. 1 + 1 HCl per 100 ml.; and 20 ml. 95 per cent ethyl alcohol per 100 ml. This solution served as a standard in the determination of the effects of the various ions and HCl upon the apparent p.p.m. of calcium, potassium and sodium. Other groups of solutions were prepared having the same ion concentrations as this standard except for one element which varied in concentration, as for example, from 0 to 24 p.p.m. for calcium, 0 to 8 p.p.m. for sodium and 0 to 24 p.p.m. for potassium.

In the concentration ranges studied magnesium did not affect the intensity of calcium, potassium nor sodium. Phosphorous as phosphate likewise did not affect the determination of sodium or potassium, but did have a marked effect upon calcium (fig. 1). This effect is a function of concentration up to 7 p.p.m. phosphorus but becomes constant for phosphorus concentrations above 7 p.p.m.;

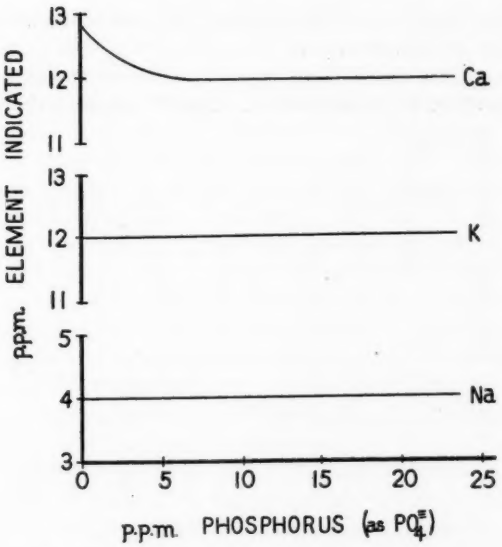

FIG. 1. Effect of phosphorus upon the apparent p.p.m. of the elements indicated.

hence the effect of phosphorus upon calcium was reduced to a neglible amount by incorporating phosphorus in all standard solutions in an amount greater than 7 p.p.m. Fig. 2 shows a similar effect of HCl upon potassium. Here again,

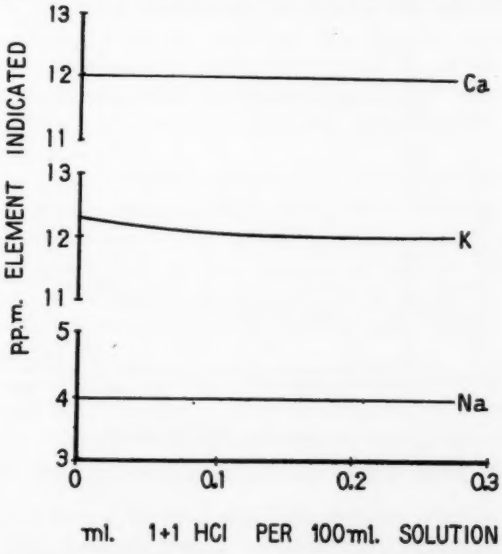

FIG. 2. Effect of HCl upon the apparent p.p.m. of the elements indicated.

the effect was minimized by maintaining the HCl concentration at or above 0.15 ml. 1 + 1 HCl per 100 ml. solution.

Although potassium and sodium have no effect upon the calcium intensity, they do have an effect upon each other. Fig. 3 indicates the effect of sodium

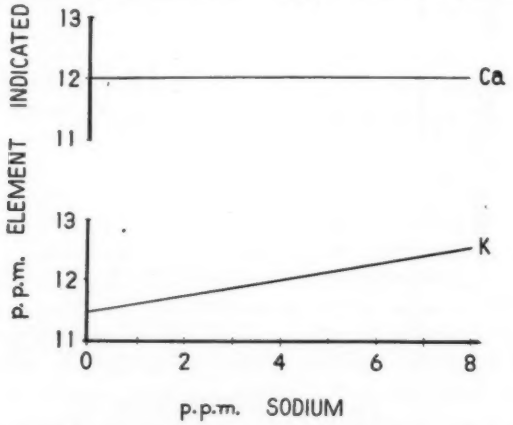

FIG. 3. Effect of sodium upon the apparent p.p.m. K and Ca.

upon potassium and fig. 4, the effect of potassium upon sodium. In both cases, the effect varies over the whole concentration range investigated, hence a correction must be applied in each case unless the unknown solution contains the same concentration of these two ions as the standards.

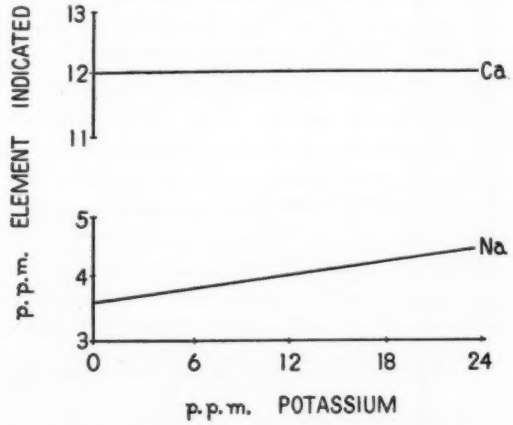

FIG. 4. Effect of potassium upon the apparent p.p.m. Ca and Na.

Sodium was added to each standard at a concentration of 4 p.p.m. Potassium was added at a 12 p.p.m. concentration. Hence, for each p.p.m. potassium difference from 12 p.p.m. in the unknown, a correction of 0.03 p.p.m. sodium was

made for the sodium concentration. This correction was added if the concentration of potassium were above 12 p.p.m. and subtracted if the concentration were below 12 p.p.m.

Similarly, a correction for potassium was necessary when the sodium concentration differed from 4 p.p.m. A correction of 0.13 p.p.m. potassium was made for each p.p.m. sodium difference from 4 p.p.m. The correction was added when the sodium concentration in the unknown was greater than 4 p.p.m. and subtracted when sodium was below 4 p.p.m.

Theoretically, a determination involving an intercorrection of this sort is not a good one. In routine milk analysis, however, where the sodium and potassium concentrations varied between relatively narrow limits, by establishing the sodium concentration in the potassium standards at 4 p.p.m. and the potassium concentration in the sodium standards at 12 p.p.m. (the approximate concentrations of these two elements in the diluted milk samples), the correction due to the effects of either of these two ions on the other was small, and, in the majority of cases, within the experimental error.

Preparation of sample stock solutions. The milk to be analyzed was warmed to approximately 21° C., mixed thoroughly by pouring from one container into another and a 100-g. sample weighed accurately into a 300-ml. porcelain crucible. One-half ml. glacial acetic acid was added to prevent the formation of surface film during drying and the sample dried at 100° C. for 24 hr. When dry, the sample was placed in a muffle furnace and the temperature gradually raised to 550° C. After cooling, the ash was dissolved by leaching for 30 min. with 10 ml. of 1:1 HCl and approximately 100 ml. of hot water. This solution then was filtered through ashless filter paper into a 500-ml. volumetric flask. The filter paper was returned to the porcelain crucible and the ashing, leaching and filtering were repeated to insure complete ashing and extraction. Following the second filtration, the filtered solution was made to 500 ml. with distilled water. Aliquots of this solution were used for analysis. This solution will be referred to as the sample stock solution.

Preparation of standard stock solutions. A sodium stock solution was prepared by dissolving reagent grade NaCl in distilled water to give a solution containing 4 mg. sodium per ml. A potassium stock solution was prepared by dissolving reagent grade KCl in water to give a solution containing 12 mg. potassium per ml. A calcium stock solution was prepared by dissolving pure $CaCO_3$ in dilute HCl, warming and diluting with water to give a concentration of 12 mg. calcium per ml.

In addition, a solution containing phosphate was prepared from syrupy phosphoric acid (85 per cent) to give an approximate concentration of phosphorus of 12 mg. per ml. The exact phosphorus concentration was determined chemically, using the ammonium molybdate method of Pemberton (21) and Kilgore (13) and modified by Hibbard (11).

Preparation of solutions to be atomized into flame. The unknown solutions atomized into the flame were prepared by pipetting 5-ml. aliquots of the sample stock solution into a 100-ml. volumetric flask, adding 20 ml. of 95 per cent ethyl

alcohol and diluting to the mark with distilled water. This solution was used for the determination of calcium, sodium and potassium.

Standard solutions atomized into the flame were prepared from aliquots of the standard stock solutions to give solutions with the following compositions:

Calcium standards

Calcium	8, 11, 14, 17 p.p.m.
Phosphorus	12 p.p.m.
Hydrochloric Acid (1 + 1)	0.2 ml. per 100 ml. solution
Ethyl Alcohol (95%)	20 ml. per 100 ml. solution

Potassium standards

Potassium	7, 10, 12.5, 15, 17 p.p.m.
Sodium	4 p.p.m.
Hydrochloric Acid (1 + 1)	0.2 ml. per 100 ml. solution
Ethyl Alcohol (95%)	20 ml. per 100 ml. solution

Sodium standards

Sodium	2, 4, 5, 6.5 p.p.m.
Potassium	12 p.p.m.
Hydrochloric Acid (1 + 1)	0.2 ml. per 100 ml. solution
Ethyl Alcohol (95%)	20 ml. per 100 ml. solution

Instrument settings used for each of the three elements determined are tabulated below:

	Calcium	*Sodium*	*Potassium*
Oxygen pressure (*in. of water*)	48	40	52
Air pressure (*p.s.i.*)	28	28	28
Gas pressure (*cm. n-butyl alcohol*)	3.5	3.5	3.5
Wavelength ($m\mu$.)	554	589	769
Slit width (*mm.*)	0.3	0.05	0.2
Phototube used	ultraviolet	ultraviolet	red
Sensitivity:			
Switch	0.1	0.1	0.1
Knob	3 turns from counter clockwise limit	2 turns from counter clockwise limit	5 turns from counter clockwise limit

Standard curves, fig. 5, were prepared as a guide for the choice of standards to be used in the determination of the unknowns. The solutions to be analyzed were handled in the following manner: Clean, dry, 5-ml. beakers were filled with the unknown solution and appropriate standard solutions. After the proper instrument settings were made and the spray chamber heated to operating temperature, the unknown was placed in position, atomized into the flame

and the relative intensity of the desired element determined. By referring this intensity to the standard curve, a choice of two standards was made such that one was of slightly lower, the other of slightly higher concentration than the unknown. The relative intensities of the ion in each of these two standards were quickly determined merely by replacing the beakers, balancing the circuit to the galvanometer null point and taking the intensity reading. Finally, the unknown was replaced on the instrument to check the value of its first reading, thereby checking the consistency of operation during the series of readings. If a variation greater than 0.2 scale division existed between the two readings of the unknown solution, the series of readings of the unknown and two standards was repeated until no variation existed between the first and second reading of the unknown. This manner of reading was preferred to that of averaging sev-

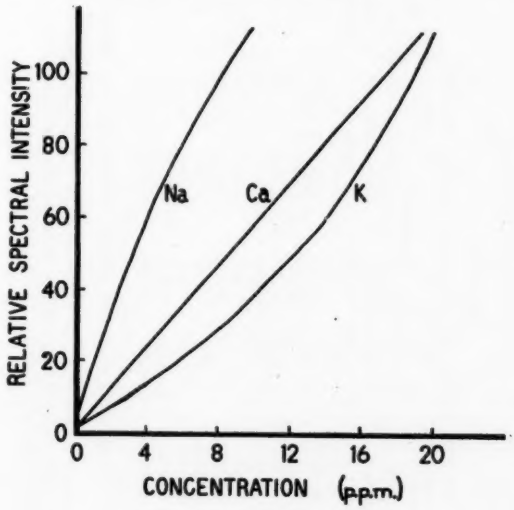

FIG. 5. Standard curves.

eral readings on each solution in that it lessened errors due to drifting and partial capillary clogging and generally required a shorter time. The remaining unknown solutions were handled in a similar manner to determine the same element. The instrument was reset for the determination of the second element and all unknown solutions determined. Finally, the group of unknowns was analyzed for the third element. The authors determined a group of twenty unknowns for three elements in duplicate in this fashion in 4 hr. including calculating time, but excluding time for sample and standard solution preparation.

Results were calculated in a similar way for the three elements determined. It was assumed that the standard curve over the small range between the two standards was a straight line in each case. This was true for calcium, but not strictly true for sodium or potassium (fig. 5). However, the error introduced

TABLE 1

Per cent sodium, potassium and calcium in milk

Sample	Sodium				Potassium				Calcium			
	By flame spectrophotometer method		By chemical method[a]		By flame spectrophotometer method		By chemical method[b]		By flame spectrophotometer method		By chemical method[c]	
1	0.0367	0.0371	0.0371	0.0376	0.111	0.112	0.112	0.112	0.111	0.112	0.112	0.112
2	0.0581	0.0577	0.0592	0.0590	0.106	0.106	0.107	0.106	0.106	0.106	0.107	0.106
3	0.0379	0.0386	0.0382	0.0379	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105
4	0.0409	0.0405	0.0396	0.0416	0.123	0.122	0.122	0.122	0.123	0.122	0.122	0.122
5	0.0405	0.0404	0.0404	0.0409	0.109	0.110	0.108	0.110	0.109	0.110	0.108	0.110
6	0.0496	0.0492	0.0496	0.0498	0.118	0.119	0.118	0.118	0.118	0.119	0.118	0.118
7	0.0358	0.0364	0.0360	0.0372	0.131	0.133	0.131	0.131	0.131	0.133	0.131	0.131
8	0.0443	0.0445	0.0437	0.0448	0.122	0.123	0.122	0.122	0.122	0.123	0.122	0.122
9	0.0480	0.0485	0.0493	0.0469	0.104	0.104	0.104	0.104	0.104	0.104	0.104	0.104
10	0.0404	0.0408	0.0413	0.0401	0.120	0.120	0.120	0.120	0.120	0.120	0.120	0.120

[a] Sodium was determined gravimetrically by precipitating with zinc uranyl acetate after the removal of interfering phosphate ion with zinc carbonate (1, 18).

[b] Potassium was determined volumetrically by a modification proposed by Bray (5) of the original Koninch (14) cobaltinitrite method.

[c] Calcium was determined by precipitation as the oxalate from a slightly acid solution followed by a permanganate titration of the precipitate. This method is given by Hillebrand and Lundell (12) and modified by Meloche, *et al.* (15).

by this assumption was negligible when the standards used for the determination were not too different in concentration so that the small segment of the curve could be assumed a straight line. Hence, if C_x, C_1, and C_2 are the concentrations of the unknown, the standard solution of lower concentration and the standard of higher concentration, respectively, and I_x, I_1, and I_2 are the corresponding relative intensities, then the concentration of the unknown is given by: $C_x = C_1 + \frac{(C_2 - C_1)\ (I_x - I_1)}{(I_2 - I_1)}$. Corrections for the effect of sodium and potassium on each other must be applied where necessary.

Table 1 shows results in duplicate of ten samples determined by the stated technique together with duplicate results determined chemically. The four results listed for each sample were determined from aliquots of the same solution of milk ash.

TABLE 2

Replicate determinations of the same sample

Date	Calcium		Sodium		Potassium	
	Duplicates	Av.	Duplicates	Av.	Duplicates	Av.
Feb. 22	0.1180 0.1185	0.1182	0.0461 0.0467	0.0464	0.1416 0.1405	0.1410
23	0.1174 0.1194	0.1184	0.0459 0.0467	0.0463	0.1381 0.1387	0.1384
24	0.1169 0.1189	0.1179	0.0469 0.0463	0.0464	0.1366 0.1362	0.1364
25	0.1184 0.1181	0.1182	0.0459 0.0456	0.0458	0.1378 0.1378	0.1376
27	0.1200 0.1195	0.1198	0.0466 0.0466	0.0466	0.1386 0.1371	0.1378
28	0.1190 0.1190	0.1190	0.0462 0.0464	0.0463	0.1402 0.1420	0.1411
Mar. 1	0.1197 0.1198	0.1198	0.0467 0.0465	0.0466	0.1390 0.1385	0.1385
2	0.1189 0.1194	0.1192	0.0465 0.0460	0.0462	0.1374 0.1380	0.1377
3	0.1196 0.1190	0.1193	0.0466 0.0464	0.0465	0.1403 0.1382	0.1392
4	0.1189 0.1190	0.1190	0.0463 0.0464	0.0464	0.1398 0.1393	0.1396
Av.		0.1189		0.0464		0.1387

TABLE 3

Analysis of data in table 2

	Calcium	Sodium	Potassium
Total sum of squares	0.000012	0.0000021	0.000075
Sum of squares between days	0.0000075	0.0000012	0.000041
Sum of squares within days	0.0000046	0.00000091	0.000034
Error within days	0.00000046	0.000000091	0.0000034
Error standard deviation on a particular day	0.00068	0.00030	0.0018
Per cent error	0.0057	0.0065	0.013
Number of replicate determinations necessary to give 99% accuracy	2	2	4

To determine reproducibility of this technique, one sample was chosen and the three elements determined in duplicate on each of 10 days. The results of this study are tabulated in table 2. The statistical analysis of data in table 2 is presented in table 3. The last line of table 3 gives the number of replicate determinations necessary to give 99 per cent accuracy. On the basis of duplicate determinations, potassium results were within $\pm$ 2 per cent of the amount present while sodium or calcium duplicates gave an accuracy of $\pm$ 1 per cent of the amount present. The average of the 20 replicates for each element was considered the amount present in the calculation of accuracy, since the exact amounts of the elements in the sample were unknown.

SUMMARY

A flame photometer technique suitable for single optical system instruments is described which gives excellent results for calcium, potassium and sodium in milk. The method consists essentially of determining the relative spectral intensity of the element in an unknown solution followed quickly by the determination of the intensity of the element in two standard solutions, one of slightly higher concentration, the other of slightly lower concentration than the unknown. The concentration of the unknown then is calculated by interpolation. The effects of extraneous ions upon the spectral intensity of the elements determined are minimized either by incorporating the influencing ions in the standard solutions at concentration levels similar to those found in the unknown solutions or by making appropriate predetermined numerical corrections.

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BLOOD PLASMA VITAMIN A AND CAROTENE OF DAIRY CALVES TREATED WITH SULFONAMIDES[1,2]

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INTRODUCTION

The antagonistic action of sulfonamides toward certain nutrients and metabolites has been reported (8, 9, 10, 11, 12, 18). These antagonistic effects apparently have not been considered complicating factors in the therapeutic use of drugs. A review of the available literature reveals that information concerning the effects of sulfonamide administration upon the normal utilization and physiological function of vitamin A, carotene and other compounds in the young calf is lacking. Lundquist and Phillips (12) found no apparent effect of sulfasuxidine administration on the blood vitamin A and carotene levels of calves. Further investigation with some of the commonly used drugs appeared to be desirable.

It has been demonstrated that calves at birth have a very limited reserve of vitamin A and that supplying an ample amount early in life is important (2, 4, 14, 15). A number of investigators have shown the importance of adequate supplies of vitamin A for proper growth and development and for resistance to diseases, particularly scours and pneumonia (6, 16, 17, 19, 22). It appeared to be advisable, should any indication of a disturbance of the normal vitamin A metabolism of a young calf due to administration of sulfonamides be demonstrated, that protective measures be undertaken in the routine raising of these animals. It was because of these and related problems that the present study was undertaken.

EXPERIMENTAL PROCEDURE

Experiments were designed to ascertain the effects of the administration of sulfathaladine, sulfamerazine and sulfathiazole on the blood plasma vitamin A and carotene levels of young dairy calves. Male Holstein calves were procured from various Pennsylvania State institutional herds for the study. The calves were managed under controlled conditions for 1 wk. prior to the observation period to allow for stabilization of the animals.

The calves were quartered in individual pens equipped with a waterbowl, hay rack and feed trough, in an artificially heated and ventilated experimental calf barn. The rations consisted of 8 lb. of Holstein herd milk fed twice daily, with

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[1] Taken from data presented in a thesis to the graduate faculty of The Pennsylvania State College by Magnar Ronning in partial fulfillment of the requirements for the degree of Master of Science. The authors wish to express their appreciation to Sharp and Dohme, Inc., Glenolden, Pa., for supplying the sulfonamides used in this trial.

[2] Authorized for publication as paper no. 1579 in the Journal series of The Pennsylvania Agricultural Experiment Station.

U.S. no. 1 timothy hay and the following calf starter available *ad libitum:* Ground yellow corn, 406.5 lb.; wheat bran, 300 lb.; crushed oats, 400 lb.; linseed oil meal, 140 lb.; soybean oil meal, 280 lb.; dehydrated alfalfa meal, 140 lb.; cane molasses, 100 lb.; dried skim milk, 100 lb.; dried corn distillers' solubles, 100 lb.; irradiated yeast (type 9F), 0.5 lb.; dicalcium phosphate, 10 lb.; ground limestone, 10 lb.; iodized salt, 10 lb.; cobalt sulfate, 2 g.; and vitamin A feeding oil (2,724,000 U.S.P. units per lb.), 3 lb.

Twenty apparently normal calves were subdivided into four comparable groups (groups I, II, III and IV) and treated respectively with sulfathaladine, sulfamerazine and sulfathiazole and the last group maintained as controls. The experiments were begun when the calves were 12 days of age. Forty milliliters of blood for vitamin A and carotene analysis were drawn from the jugular vein daily, 3 hr. after the morning feeding, for 13 consecutive days.

The morning of the third day, when the calves were 14 days of age, sulfonamide administration was begun. A standard dosage was administered orally to all the calves of the treated groups as follows: 10 g. after the morning bleeding on the fourteenth day, followed by three 5-g. administrations at 12 hr. intervals. Approximately 6 ml. of blood were removed 3, 6 and 12 hr. after the first administration of the drug for sulfonamide determinations. Thereafter, the blood level of the drug was determined daily. When the calves reached the age of 28 days, the procedure was repeated on each calf with the same type and dose of sulfonamide as used previously. Untreated controls were similarly fed, managed and studied.

The carotene and vitamin A determinations were made using a combination of methods by Moore (13) and Kimble (5) as modified by Knodt (7). Sulfonamide blood levels were determined according to the Bratton-Marshall method (1). All determinations were colorimetric and the per cent transmission was measured with an Evelyn photoelectric colorimeter (3).

RESULTS

The effects of the administration of sulfathaladine, sulfamerazine and sulfathiazole upon the levels of blood plasma vitamin A and carotene are summarized in tables 1 and 2. A comparison of the mean blood plasma levels for the 3 days prior to administration with those for the ninth, tenth and eleventh days after initial administration has been made. Whereas the blood plasma vitamin A of the control calves decreased 3.31 μg. per 100 ml. during the first trial, changes of -0.01, -2.67 and $+2.67$ were observed in the groups treated with sulfathaladine, sulfamerazine and sulfathiazole, respectively.

In the second trial, all groups of calves exhibited an increase in the mean blood plasma vitamin A levels, being more marked in the case of the sulfonamide-treated calves. The sulfathaladine-treated calves reacted similarly to those of the control group, but treatment with sulfamerazine and sulfathiazole resulted in greater increases in plasma vitamin A as compared to the controls. The greatest increase occurred with the administration of sulfamerazine.

Somewhat similar results were obtained with respect to the effects of those sulfonamides studied upon the levels of blood plasma carotene. With the exception of the calves constituting the sulfamerazine-treated group, the greatest decline in plasma carotene was exhibited by the control group. The decline observed in the case of group II might be attributed to the extremely high initial blood plasma carotene level of this group. In the second trial, all the sulfonamide-treated groups showed much greater increases in the carotene blood plasma

TABLE 1

Mean daily blood plasma vitamin A concentrations of each group of calves (μg./100 ml.)

Day of administration	Group I sulfathaladine	Group II sulfamerazine	Group III sulfathiazole	Group IV control
		First trial		
−1	11.03	17.91	10.85	14.68
0	10.68	18.25	11.50	15.91
1	9.68	15.57	10.86	14.46
2	8.92	15.57	10.29	13.38
3	9.01	14.07	10.47	13.57
4	10.20	13.67	11.23	13.13
5	10.41	14.24	12.96	13.46
6	10.16	14.91	12.72	12.80
7	9.75	14.82	12.27	12.49
8	9.63	16.74	11.66	12.10
9	9.91	15.32	14.30	12.52
10	10.27	15.08	14.27	11.30
11	11.16	13.30	12.64	11.30
$\bar{X}$	10.06	15.34	12.00	13.16
Change[a]	−0.01	−2.67	+2.67	−3.31
		Second trial		
−1	11.94	11.19	11.35	10.04
0	9.52	9.67	11.30	10.83
1	9.26	8.95	10.30	10.66
2	9.27	9.55	10.60	10.77
3	9.52	11.07	9.40	10.13
4	10.33	12.71	11.88	10.20
5	11.08	12.49	12.88	11.33
6	10.91	10.51	12.57	10.32
7	11.30	11.77	13.35	10.69
8	11.30	12.08	12.02	10.87
9	10.45	11.94	12.32	11.53
10	10.99	12.60	12.85	11.23
11	11.22	11.80	12.49	10.20
$\bar{X}$	10.55	11.26	11.79	10.68
Change[a]	+0.65	+2.17	+1.57	+0.48

[a] A comparison of the −1, 0 and 1 with the 9, 10 and 11 d. of the trial.

levels than the control groups. It must be emphasized that in certain instances there were large variations in the response of the individual calves.

A summary of the free and total blood levels of the various sulfonamides used is presented in table 3. The highest levels were observed on the third day of administration in all cases. Sulfamerazine apparently was the most readily absorbed of the sulfonamides, while sulfathiazole was retained for a longer period. Sulfathiazole also exhibited the greatest degree of acetylation, as indicated by difference in the amount of the free and the total form of the drug

found in the blood. Although sulfathaladine was not absorbed to a very large extent, it apparently was acetylated at a rather high rate. Nearly all of the sulfamerazine detected in the blood was in the free form. In some instances, where low concentrations of blood sulfamerazine were observed, the difference between free and total sulfamerazine values was less than variations which might reasonably be attributed to laboratory techniques.

There was an increased absorption noted in the case of all the sulfonamides during the second trial and sulfamerazine and sulfathiazole apparently were

TABLE 2

Mean daily blood plasma carotene concentrations of each group of calves (μg./100 ml.)

Day of administration	Group I sulfathaladine	Group II sulfamerazine	Group III sulfathiazole	Group IV control
		First trial		
−1	11.46	35.39	9.13	29.48
0	10.69	34.12	8.36	26.46
1	11.07	29.73	7.77	24.59
2	9.13	26.68	7.77	21.62
3	11.08	23.26	7.97	20.90
4	10.69	19.46	8.16	21.74
5	9.52	17.88	9.91	20.07
6	10.10	17.90	11.27	22.37
7	9.72	18.46	10.88	21.21
8	9.52	19.04	10.32	20.62
9	10.30	19.04	10.49	20.40
10	10.49	17.68	10.30	19.63
11	10.30	17.29	12.44	20.21
$\overline{X}$	10.31	22.76	9.60	22.25
Change[a]	−0.71	−15.08	+2.66	−6.76
		Second trial		
−1	14.38	24.21	10.50	17.10
0	13.99	24.21	10.30	15.94
1	13.60	20.99	10.30	15.16
2	13.99	19.08	11.08	14.57
3	14.77	21.57	11.27	16.71
4	15.74	24.79	12.05	18.07
5	17.29	27.12	14.77	16.13
6	17.29	27.12	14.57	15.54
7	18.07	29.59	14.77	16.52
8	19.43	27.31	14.38	15.94
9	19.82	26.90	15.16	17.29
10	21.18	27.70	15.16	18.10
11	22.35	28.59	14.77	17.29
$\overline{X}$	17.07	25.32	13.01	16.49
Change[a]	+7.13	+4.59	+4.66	+1.49

[a] A comparison of the −1, 0 and 1 with the 9, 10 and 11 d. of the trial.

absorbed at a higher initial rate. Sulfathaladine, however, exhibited a lower rate of initial absorption during this period as compared to the first trial.

DISCUSSION

It appears that under the conditions of these experiments, calves which had been treated with sulfonamides maintained blood plasma concentrations of vitamin A and carotene at comparable levels with those observed in the blood of control animals.

The relative rates and degrees of absorption of the various sulfonamides were found to be in agreement with the reports of other investigations (20, 21).

SUMMARY

Observations were made to determine possible effects of sulfonamide administration upon the blood plasma vitamin A and carotene levels of young calves.

On the basis of these data, it does not appear that sulfonamide therapy has any detrimental effects on the normal vitamin A and carotene metabolism of calves when the drugs are administered as recommended.

TABLE 3

Mean free and total sulfonamide blood concentrations of each group of calves from 3 hr. after first administration to end of period (mg./100 ml.)

Day	Hour	Group I sulfathaladine		Group II sulfamerazine		Group III sulfathiazole	
		Free	Total	Free	Total	Free	Total
				First trial			
1	3	0.14	0.23	0.64	0.62	0.58	0.63
	6	0.31	0.56	2.69	2.61	1.44	1.69
	12	0.53	0.92	4.70	4.72	1.93	2.37
2		0.58	1.09	8.14	8.49	2.88	3.57
3		0.68	1.24	12.14	12.81	4.09	6.19
4		0.62	1.05	11.87	12.77	2.86	4.35
5		0.36	0.68	9.65	10.23	1.95	4.30
6		0.27	0.41	6.01	10.75	0.82	2.04
7		0.09	0.18	2.73	2.84	1.03	3.87
8		0.05	0.10	1.40	1.49	0.55	2.14
9		0.06	0.14	1.18	1.29	0.50	2.08
10		0.04	0.12	0.23	0.24	0.10	0.30
11		0.01	0.04	0.28	0.31	0.18	1.09
				Second trial			
1	3	0.02	0.07	1.88	2.10	0.84	0.98
	6	0.10	0.25	6.09	6.03	1.51	1.88
	12	0.36	0.69	8.38	8.33	1.99	2.91
2		0.66	1.11	10.70	9.91	3.66	5.24
3		1.20	1.83	14.49	13.80	5.22	8.19
4		0.88	1.40	8.24	8.87	3.64	6.91
5		0.24	0.51	2.82	4.17	2.86	6.20
6		0.10	0.26	0.62	1.65	2.48	6.00
7		0.11	0.23	0.16	0.44	1.92	5.03
8		0.06	0.63	0.06	0.10	1.41	4.39
9		0.06	0.16	0.00	0.00	0.92	3.42
10		0.01	0.10	0.00	0.00	0.72	3.08
11		0.03	0.14	0.00	0.00	0.43	2.61

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MOTILITY OF BOVINE SPERMATOZOA IN BUFFERED WHOLE EGG

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The cost of ingredients and labor going into the preparation of yolk extenders for bovine semen is considerable when large quantities are required. Swanson (8) has reported satisfactory results with an extender consisting of three parts citrate buffer and one part egg yolk. Thus, substantial savings appear to be possible by reducing the amount of egg yolk used. If the whole egg (yolk plus albumen) could be utilized, even more savings would be expected, since the volume of yolk plus albumen from an egg is approximately three times the volume of the yolk itself.

At the time that Phillips and Lardy (4) reported the use of buffered liquid egg yolk as a satisfactory medium in which to store bovine spermatozoa, they also reported that egg yolk or egg albumen used alone was not satisfactory. Furthermore, they reported the optimum pH range for the storage of bovine spermatozoa to be 6.7 to 6.8 and the pH of egg yolk to be appoximately 6.0. Therefore, a suitable buffer to adjust the pH of the yolk was indicated. The fact that whole egg and egg albumen have pH values of 7.5 to 7.8 and 8.0 to 9.0, respectively, (2, 5) suggests that the unsatisfactory results reported from the use of egg white alone (4) may have been, in part, a consequence of its pH. However, to date, the use of whole egg with a suitable buffer has not been reported. This paper reports the results of laboratory investigations undertaken to utilize liquid whole egg instead of egg yolk in extenders for bovine semen.

EXPERIMENTAL

The liquid whole egg was prepared by emptying the contents of alcohol-cleaned eggs into a sterile beaker, removing the chalazae with sterile forceps and mixing the whole egg material for 2 min. in a Waring blender. After allowing the blended whole egg material to settle from the foam, it was poured off and immediately mixed with the buffer in the required proportions.

Initially, spermatozoan motility studies were conducted comparing the standard 2.9 per cent citrate-sulfanilamide-yolk (2.9 CSAY) extender (1) and an experimental extender composed of equal parts of 2.9 per cent citrate-sulfanilamide buffer (1) and blended liquid whole egg. Twenty samples of bovine semen were extended at the rate of 1 : 100 in these two extenders and stored at 5° C. in 3-ml. tubes filled to capacity. At the end of 2 days storage, 44 per cent of the spermatozoa were motile in the 2.9 CSAY but only 11 per cent were motile in the 2.9 per cent citrate-sulfanilamide-whole egg extender (2.9 CSAYWE). This difference was reasonable in view of the fact that the pH of the yolk extender was 6.66, while the pH of the whole egg extender was 7.82.

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On the basis of these initial observations, a series of citrate-sulfanilamide-whole egg extenders was prepared in which the pH was adjusted by the use of acetic, lactic, succinic and hydrochloric acid. In addition to these common acids, a sulfonamide with acidic properties, succinylsulfathiazole (sulfasuxidine), was used to lower the pH of the whole egg extender and, at the same time, substitute for sulfanilamide. In these extenders the proportion of buffer to whole egg was changed from 1:1 to 3:1, but the concentration of citrate and sulfonamide was maintained at the same levels as in the 2.9 CSAY, namely, 1.45 per cent sodium citrate dihydrate and 300 mg. per cent sulfonamide. Therefore, an extender having a 3:1 ratio of buffer to whole egg required a buffer containing 1.93 per cent citrate and 400 mg. per cent of sulfonamide. Twenty samples of fresh semen were extended at rates of 1:100 in these acidified whole egg extenders and compared with those same samples extended in the standard 2.9 CSAY (1). The initial pH of each extender and the per cent of motile spermatozoa at 0, 1, 2, 3 and 4 days of storage at 5° C. are shown in table 1.

TABLE 1

Per cent of motile spermatozoa in extenders containing whole egg acidified with different acids and stored at 5° C.
(Averages of 20 ejaculates from 14 bulls)

Extender[a]	Acid used	pH of extender	Days stored				
			0	1	2	3	4
2.9 CSAY	None	6.71	56	47	42	34	32
1.9 CSAWE	None	7.88	46	10	4	1	1
"	Acetic	6.58	54	41	33	29	26
"	Lactic	6.81	58	43	37	32	27
"	Succinic	6.66	56	41	36	36	32
"	HCl	6.60	58	46	39	33	30
1.9 CSSWE	None	6.67	59	49	44	37	35

[a] 2.9 and 1.9 represent the percentages of sodium citrate dihydrate in the buffer; C = sodium citrate dihydrate; SS = sulfasuxidine; SA = sulfanilamide; Y = egg yolk; WE = whole egg (yolk and albumen).

From these storage data it was obvious that adjustment of the pH was of primary importance in the utilization of whole egg. The combination of three parts of 1.93 per cent citrate containing 0.4 per cent sulfasuxidine and one part of whole egg (1.9 CSSWE) appeared to be as satisfactory for spermatozoan motility as the standard 2.9 CSAY. Since the use of the common acids for pH adjustment required one more step in buffer preparation, the use of sulfasuxidine was considered desirable.

To avoid the slight hypotonicity accompanying the use of the 1.93 per cent citrate-sulfasuxidine buffer, the 2.9 per cent citrate buffer containing 0.4 per cent sulfasuxidine was used. Each buffer was mixed in a ratio of three parts buffer to one part whole egg and compared with the standard 2.9 CSAY. Twenty fresh semen samples were extended at the rate of 1:100 in these three extenders and stored at 5° C. for 4 days. The initial pH of the extenders and the per cent of motile spermatozoa during storage are shown in table 2. During the first day

or two of storage, the 2.9 CSSWE was slightly superior to both the 1.9 CSSWE and the 2.9 CSAY control.

Another sulfonamide, succinylsulfanilamide, a derivative of sulfanilamide having acidic properties, was used to replace the succinylsulfathiazole in the 2.9 CSSWE formula. Motility of the spermatozoa appeared to be as satisfactory in the whole egg extender containing the succinylsulfanilamide (2.9 CSSAWE) as in the 2.9 CSSWE or the 2.9 CSAY.

During the course of these storage studies, it was observed that the blended whole egg and those whole egg extenders with a high pH changed within a few hours from a light yellow to a dark brown color. These color changes may have been caused by enzymatic reactions at the high pH levels (3), since the whole egg extenders adjusted to a pH of 6.6 to 6.8 retained their initial light yellow color, even when stored with spermatozoa for 30 days. Combinations of liquid whole egg with phosphate buffers have not been satisfactory because of the precipitates formed.

TABLE 2

Per cent of motile spermatozoa in yolk and whole egg extenders when stored at 5° C. (Averages of 20 ejaculates from 18 bulls)

Extenders[a]	pH of extender	Days stored				
		0	1	2	3	4
2.9 CSAY	6.72	54	52	46	43	38
2.9 CSSWE	6.75	65	56	49	37	34
1.9 CSSWE	6.68	52	44	44	40	38

[a] See footnote table 1.

DISCUSSION

The whole egg extenders cost approximately 25 per cent as much as the standard yolk extenders. Other advantages were the small amount of time required for preparation, the ease with which glassware coming in contact with them was cleaned and the more distinct appearance of the spermatozoa during microscopic examinations. Settling out, which is a problem in the mixing of samples for microscopic examination or for insemination, was less pronounced in the 1.9 CSSWE and the 2.9 CSSWE than in the 2.9 CSAY. This may be explained by the fact that sulfasuxidine is more soluble than sulfanilamide and does not crystallize out readily during storage at 5° C. in the whole egg formulae. It also was observed that, in contrast to the dark brown color developed by the citrate buffers containing sulfanilamide when exposed to sunlight (6), those buffers containing sulfasuxidine developed only a light brown color.

From the results of these studies it appears that citrate-sulfasuxidine buffered liquid whole egg may be used as an extender for bovine semen if it does not impair the fertility of the spermatozoa. Field trials to test the fertility of spermatozoa stored in citrate-sulfasuxidine buffered liquid whole egg have been conducted, the results of which are to be reported in an accompanying paper.

SUMMARY

Spermatozoan motility at 5° C. was as satisfactory in extenders composed of three parts of a 2.9 per cent sodium citrate dihydrate buffer containing 0.4 per cent of succinylsulfathiazole or 0.4 per cent succinylsulfanilamide and one part of liquid whole egg as in the standard 2.9 per cent citrate-sulfanilamide-yolk extender.

The costs of the whole egg extenders were approximately 25 per cent of that of the yolk extender. The former could be prepared more quickly and glassware coming in contact with them was much easier to clean. The spermatozoa were more visible and more active in the whole egg extenders, thereby facilitating microscopic examinations.

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FERTILITY AND MOTILITY OF BOVINE SPERMATOZOA IN BUFFERED WHOLE EGG EXTENDERS

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In previous reports from this laboratory, Dunn and Bratton (2, 3) presented preliminary data showing that satisfactory motility and fertility of bovine spermatozoa could be obtained with a citrate-sulfasuxidine buffered liquid whole egg extender. The present paper reports results of more extensive investigations on the fertility and motility of bovine spermatozoa in this type of extender.

EXPERIMENTAL

Two field trials were conducted, the first from December 28, 1948, to January 11, 1949, and the second from May 16 to June 9, 1949. In the first trial each of 69 ejaculates from 37 Holstein bulls was split two ways. One portion was extended in 2.9 per cent citrate-sulfanilamide-yolk (2.9 CSAY) and the other in 1.9 per cent citrate-sulfasuxidine-whole egg (1.9 CSSWE). In the second trial each of 145 ejaculates from 48 bulls, 31 Holsteins and 17 Guernseys, was divided into four portions and each portion extended in one of the following extenders: 3.5 CSAY, 2.9 CSAY, 2.9 CSSWE, and 1.9 CSSWE.

The composition of the buffers and extenders are shown in table 1. The buffers were prepared once a week and the extenders during the afternoon of the day prior to their use.

TABLE 1

Composition of buffers and extenders

Extenders[a]	Concentration of citrate and sulfonamides in buffers			Proportion of buffer to egg material		Concentration of citrate and sulfonamides in extenders			
	C	SA	SS	Y	WE	C	SA	SS	pH
	(%)	(%)	(%)			(%)	(%)	(%)	
3.6 CSAY	3.60	0.60	.....	1:1	.....	1.45	0.30	.....	6.78
2.9 CSAY	2.90	0.60	.....	1:1	.....	1.45	0.30	.....	6.76
2.9 CSSWE	2.90	.....	0.40	.....	3:1	2.175	.....	0.30	6.75
1.9 CSSWE	1.93	.....	0.40	.....	3:1	1.45	.....	0.30	6.70

[a] 1.9, 2.9 and 3.6 represent the per cent of sodium citrate dihydrate in the buffer. C = citrate; SA = sulfanilamide; SS = sulfasuxidine; Y = egg yolk; WE = whole egg.

In both trials each portion of each ejaculate was partially extended (1:4) in its respective extender immediately after collection, cooled slowly from approximately 30 to 5° C. during a period of 55 min. and then made up to the final volume required with cold (5° C.) extender. Only semen samples initially con-

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taining 500×10^6 or more spermatozoa per ml. and 50 per cent or more motile spermatozoa were used for insemination. Final extension rates averaged 1 : 68 in the first trial and 1 : 86 in the second trial, the average number of motile spermatozoa per milliliter of extended semen being 17×10^6 and 14×10^6, respectively, in the two trials. Microscopic examinations of all extended semen samples were made at intervals of 0, 1 and 3 days of storage at 5° C.

The fertility of the semen was based on 60- to 90-day non-returns to first and second service cows and expressed as per cent non-returns. Analysis of variance (7) was used to test the statistical significance between the means, the ejaculate × extender sub-class percentages being the experimental units.

RESULTS AND DISCUSSION

In table 2 are the estimated percentages of motile spermatozoa during storage at 5° C. in the different extenders. The per cent of motile spermatozoa compared favorably with previous findings (2, 3). The rate of progressive movement of the spermatozoa was difinitely superior in the whole egg extenders. While theoretically the 1.9 CSSWE was slightly hypotonic (6), the motility of spermatozoa in this extender appeared to be as satisfactory as in the 2.9 CSSWE and the yolk extenders.

TABLE 2

Average per cent of motile spermatozoa in citrate-yolk and citrate-whole egg extenders when stored at 5° C.

	Days of storage	Extenders[a]			
		3.6 CSAY	2.9 CSAY	2.9 CSSWE	1.9 CSSWE
1st trial	0		61		62
(av. of 69 ejac. from 37 bulls)	1		56		60
	3		49		53
2nd trial	0	66	64	69	65
(av. of 145 ejac. from 48 bulls)	1	60	60	64	63
	3	53	56	51	57

[a] See footnote table 1.

In table 3 are the total number of first- and second-service cows inseminated in the two trials and the per cent 60- to 90-day non-returns to these cows. In both trials the per cent non-returns for the first- and second-service cows combined averaged slightly higher for the 2.9 CSAY than for the 1.9 CSSWE extender. In the second trial the per cent non-returns averaged slightly higher for the 2.9 CSAY than for the 2.9 CSSWE. On the other hand, the average non-return percentages for first- and second-service cows combined were slightly higher than they were for the 3.6 CSAY formula. Yet, none of the differences between the means for the different extenders were statistically significant ($P < 0.05$).

In the two trials, 10,923 first- and second-service cows were inseminated with the CSAY formulae and 11,289 with the CSSWE formulae. On the basis of the 60- to 90-day non-returns, the yolk formulae (3.6 CSAY and 2.9 CSAY) averaged

62.3 per cent and the whole egg formulae (1.9 CSSWE and 2.9 CSSWE) averaged 61.6 per cent. Therefore, it appears that 2.9 CSSWE may be used as an extender for bovine semen with the expectation of fertility results practically as good as those obtained by the use of 2.9 CSAY extender.

In view of the favorable results on fertility levels reported by Almquist (1), Easterbrooks *et al.* (4) and Foote *et al.* (5) when penicillin and streptomycin have been used in the citrate-sulfanilamide-yolk extender, the question arises as to whether or not the addition of these antibacterial agents to the whole egg extender will be accompanied by similar increases in fertility. Fertility studies are being initiated to compare yolk and whole egg extenders containing penicillin, streptomycin and a sulfonamide.

TABLE 3

Fertility data

(Average per cent 60–90-day non-returns to 1st and to 1st and 2nd service cows combined)

	Extenders[a]							
	3.6 CSAY		2.9 CSAY		2.9 CSSWE		1.9 CSSWE	
	No. of serv.	N. R.[b]	No. of serv.	N. R.	No. of serv.	N. R.	No. of serv.	N. R.
		(%)		(%)		(%)		(%)
1st trial:								
1st service cows	.....	.....	2,926	59.9	.....	.....	2,987	59.2
1st & 2nd service cows combined	.....	.....	4,144	57.6	.....	.....	4,336	56.6
2nd trial:								
1st service cows	2,543	64.4	2,614	66.7	2,657	66.1	2,549	64.3
1st & 2nd service cows combined	3,370	63.9	3,409	66.0	3,517	64.5	3,436	64.2

[a] See footnote table 1.

[b] N. R. = non-returns.

SUMMARY

By means of the split sample technique, comparisons were made between the standard 2.9 and 3.6 per cent citrate-sulfanilamide-yolk extenders and whole egg extenders composed of three parts of either a 1.9 or a 2.9 per cent citrate buffer, containing 400 mg. per cent of succinylsulfathiazole and one part of blended liquid whole egg (yolk + albumen).

On the basis of approximately 11,000 first- and second-service cows inseminated artificially with each of these two types of extenders, the 60- to 90-day non-returns averaged 62.3 per cent for the citrate-sulfanilamide-yolk formulae and 61.6 per cent for the citrate-succinylsulfathiazole-whole egg formulae. Spermatozoan motility was satisfactory in both types of extenders.

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ADAPTATION OF THE TYRAMINE METHOD TO ROUTINE CHEESE ANALYSIS[1]

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In a previous paper (3) the authors presented data on the tyramine concentration of various cheeses. The method used was one applied from the studies of Bellamy and Gunsalus (1) on bacterial metabolism. Although the results obtained on cheese were accurate, the method was not conducive to large-scale operations. This was due to pre-extraction of the cheese fat and to formation of emulsions in the extraction tube produced by rapid rates of extraction. As a result, slow extraction rates were used with 48 hr. being considered average extraction time for a sample of cheese.

Recently several changes have been introduced to speed up and simplify the method. These changes include elimination of the extraction of fat, greater dilution of cheese solutions to minimize emulsion formation and changes in quantities of cheese and color reagents. The modified method for cheese is presented in this paper.

PROCEDURE

Reagents[2]. (a) Sodium carbonate solution (2 per cent). Dissolve 2 g. of sodium carbonate (anhydrous) in water and make up to 100 ml. (b) Sulphuric acid solution (*M*/50). Dilute 1.1 ml. of concentrated sulphuric acid to 1 l. (c) Phenolpthalein (1 per cent). Dissolve 1 g. phenolpthalein powder in a small quantity of 95 per cent alcohol and bring to 100 ml. with alcohol. (d) Ethyl ether, USP. (e) Acetic acid (95 per cent). (f) Mercuric sulphate-sulphuric acid solution. Dissolve 20 g. of mercuric sulphate in 190 ml. of water to which 10 ml. of concentrated sulphuric acid have been added. (g) Sodium nitrite 1.5 per cent. Dissolve 750 mgm. of $NaNO_2$ in 50 ml. of water. Use fresh daily. (h) Whatman filter paper, no. 42, 15 cm. (i) Apparatus.[3] Refer to Kosikowsky and Dahlberg (3).

Preparation of sample. Using a mortar, 2.5 g. of cheese were ground to a paste with a small amount of warm (50° C.) water. Later, more warm water was added and the mixture was transferred quantitatively to a 250-ml. volumetric flask where the level of water was brought to approximately two-thirds full. This mixture then was heated in a water bath to 85° C. and, without holding, was cooled to about 25° C. The flask was filled to its mark with water, stoppered and inverted several times. The mixture was filtered through large fluted

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[1] This investigation was aided by a grant from the National Cheese Institute.
[2] Unless otherwise specified, C.P. chemicals and distilled water were used.
[3] The complete apparatus can now be obtained from Will Corporation, Rochester, N. Y. Receiver tube will have a 6-ml. graduation on lower portion.

Whatman no. 42 paper and when enough of the relatively clear filtrate was obtained, 25-ml. samples were transferred to extraction tubes.

Extraction of cheese filtrate. The 25 ml. of cheese filtrate were made slightly alkaline by adding from 0.2 to 0.8 ml. of 2 per cent sodium carbonate solution (No phenolphthalein actually was added to the sample used in the determination, but the amount of alkali required was determined on a duplicate sample of cheese filtrate to which two drops of phenolphthalein were added). Five ml. of $N/50$ sulphuric acid were placed in the receiver tube, the glass thimble placed in the extractor tube and ethyl ether carefully added to both tubes. The two sections then were connected by means of a cork and attached to a water condenser. Immersion of the ends of the extractor tubes in an oil bath at 65 to 70° C. provided sufficient heat to force extraction at the rate of 70 to 80 drops per min. and the average cheese generally was complete in 18 to 24 hr. Preextraction of the fat under acid condition was not required in this method, as the major portion of the fat was removed by filtration in preparing the sample and the sample also was more dilute.

After extraction, the receiver tube was disengaged, placed into the oil bath and the ether was carefully boiled off. The remaining acid solution was cooled to 25° C., the volume brought up to the 6-ml. graduation lines with dilute sulphuric acid and agitated.

Development and measurement of color. Two ml. of the acid solution containing the tyramine were pipetted into a colorimeter tube (calibrated 18×150 ml. pyrex test tube). Three ml. of acetic acid were added, followed by 2 ml. of the mercuric sulphate-sulphuric acid reagent and the tube was well agitated. This mixture was heated for 3 min. in boiling water and cooled to room temperature. The tube was placed in a Coleman no. 11 spectrophotometer and was read at 500 γ against a reagent blank set at G = 100. After the turbidity reading (L_1), if any, was recorded, 0.2 ml. of fresh 1.5 per cent sodium nitrite was added and well mixed. After 15 min. at room temperature, the tube was read against a reagent blank. This second reading was labeled L_2.

The quantity of tyramine can be calculated by using a constant or it can be observed by comparison with a standard curve. Both methods were illustrated by the authors in their previous paper (3). It should be pointed out here that in making calculations the 25 ml. of cheese filtrate contains 0.25 g. of cheese and that as 2 ml. out of 6 ml. are tested, the factor to convert back to the tyramine-per-gram basis would be 12.

EXPERIMENTAL RESULTS

A number of cheddar cheeses were analyzed for tyramine by the method previously used by the authors and by the method presented here. Table 1 showing the results on ten of these cheeses indicates that the recovery of tyramine from cheese is not reduced by the introduction of these changes.

The possibility of extracting dry cheese, using Soxhlet type fat extraction units, also was explored. Extractions of this nature should be a great deal faster and simpler. Bailey-Walker extractors with a variety of siphon cups and

extraction thimbles were used. A series of cheeses was ground with either anhydrous sodium carbonate or anhydrous sodium sulphate in amounts sufficient to bind all the water in the cheese. The pH of the dry cheese with sodium carbonate had the correct pH for tyramine extraction, while the cheese mixed with sodium sulphate was on the slightly acid side. Upon extraction with ether, using various types of glass and paper extracting thimbles, both cheeses gave large amounts of color. As tyramine would not have been extracted from an acid cheese, it was apparent that through some mechanical defect either tyramine, tyrosine or some water soluble protein was coming over into the test solution. This was confirmed further by the fact that when cheeses extremely low in

TABLE 1

The tyramine concentration of cheddar cheese using the original tyramine method and the modified tyramine method

Cheeses	Original tyramine method (direct extraction of cheese)	Modified tyramine method (extraction of water extract)
	(γ tyramine/g. cheese)	(γ tyramine/g. cheese)
1	184	188
		178
2	440	459
		426
3	294	260
		260
4	701	718
		685
5	82	76
		78
6	308	311
		313
7	687	718
		695
8	104	102
		102
9	441	400
		415
10	874	880
		880

tyramine and high in tyrosine were analyzed, there was a carry-over. When applied to wet extraction, using the regular extraction apparatus, this condition did not exist. It was apparent that dry extraction of cheeses for tyramine under these conditions was not feasible.

DISCUSSION

Several changes in procedure have been advocated to improve the method of determining tyramine in cheese. The tendency for emulsions to form in the extraction tube and thus hamper the removal of tyramine has been reduced but not eliminated. For certain types of cheddar cheese these emulsions still persist and can only be handled with care as advocated earlier by the authors. The complete elimination of these emulsions would improve the method tremendously.

Attempts made in this laboratory to successfully eliminate this condition by the use of various extraction technics and by the use of protein precipitation agents have met with little success. The precipitating agent which showed the greatest promise in this approach, as pointed out by Gurdian (2), was zinc sulphate. However, although certain specific concentrations of zinc sulphate completely eliminated emulsion formation during the time required for running this test, there was a definite loss in recovery of tyramine which, though averaging only 5 per cent, was not constant.

In addition to the lessened tendency for emulsions to form, these changes have provided advantages of less handling of equipment, shorter operational time and easier computations. During the past year, over 200 samples were analyzed successfully using these changes in routine work.

In the last paper (3) on tyramine determination of cheese, sodium nitrite had been inadvertently printed as sodium nitrate. The latter, if used, will not produce color in the presence of tyramine. The present paper contains the corrected term, sodium nitrite ($NaNO_2$).

SUMMARY

The determination of tyramine in cheese has involved a long tedious procedure. A number of changes have been made to shorten the method without loss of accuracy. These include elimination of initial fat extractions, greater dilution of cheese solutions to reduce emulsion formation, more rapid extraction and use of different quantities of cheese and reagents. These changes make this method more adaptable for routine analysis of cheese.

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LIVE SPERMATOZOA RELATIONSHIPS AND FERTILITY OF DAIRY BULL SEMEN[1]

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A criterion for selecting semen samples to be used for artificial insemination which has a high correlation with the fertilizing capacity of the semen would be of great value. For the differentiation of live and dead spermatozoa, Lasley *et al.* (3) proposed a staining method employing opal blue and water soluble eosin as the dyes. Lasley and Bogart (2) employed this differential stain in a study of artificial breeding in beef cattle. They reported a coefficient of correlation of $+0.41^{**}$[3] between the percentage of live spermatozoa and the motility rating of semen samples and a correlation of $+0.83^{**}$ between fertility and the percentage of live spermatozoa in diluted semen following a 0° C. cold shock for 10 min. They also reported that semen samples containing less than 50 per cent live spermatozoa were of questionable fertilizing capacity, whereas samples containing 50 to 90 per cent live spermatozoa showed no differences in fertilizing capacity.

Madden *et al.* (4) have used the opal blue-eosin stain in other semen studies.

Schaeffer and Almquist (7, 8) reported on a differential stain employing aniline blue and eosin in the staining mixture. Coefficients of correlation between the percentage of live spermatozoa initially in semen samples and their fertilizing capacity were not sufficiently high for prediction purposes.

Mayer *et al.* (5) also have reported on a variation of the opal blue-eosin stain, substituting fast green FCF for the opal blue in the staining mixture.

The purpose of the experiments reported here was to establish further the relationships existing between certain semen characteristics (including the percentage of live spermatozoa in semen samples initially and following cold shock) and fertility, with a view to the use of these relationships as criteria for selecting semen samples for artificial insemination.

METHODS

This study was made on semen collected from 19 dairy bulls (14 Holsteins and 5 Guernseys) in a stud at the Dairy Research Farm, New Jersey Agricultural Experiment Station, Sussex. Inseminations were made by the technicians of the Sussex County Cooperative Breeding Association, Inc., in their local cooperative. Data were collected on 236 semen samples, among which were 81

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[3] Throughout this paper * represents significance at the 5 per cent point and ** represents significance at the 1 per cent point.

from 14 bulls to which 20 or more first- and second-service cows were bred for a total of 2,753 inseminations. Fertility data are based on 60- to 90-day nonreturns to service. Semen samples that were used for inseminating purposes were selected on the basis of initial motility rating and the previous breeding efficiency of the bulls.

The semen samples were collected by means of an artificial vagina and processed immediately. Spermatozoa concentration was determined turbidometrically (Salisbury *et al.*, 6) with a Klett-Summerson photoelectric colorimeter. Motility ratings were made initially on undiluted semen according to the procedure of Herman and Swanson (1) and Swanson and Herman (10). Motility ratings were made daily until motility ceased, both on the undiluted semen and on a sample of semen diluted one part to ten parts of an egg yolk-citrate diluter containing 0.3 per cent sulfanilamide. The storage temperature was 5° C. Using the fast green FCF-eosin Y differential stain (Mayer *et al.*, 5), an initial determination of the percentage of live spermatozoa was made on each semen

TABLE 1

Characterization of 236 semen samples from 19 bulls

	Mean	Standard error	Standard deviation	Range	Coefficient of variation[a]
					(%)
Undiluted semen					
Sperm concentration (*millions/ml.*)	1,388.84	31.00	476.23	542–3,025	34.28
Semen volume (*ml.*)	4.33	0.10	1.56	1.6–12.7	36.03
Sperm per ejaculate (*billions*)	6.17	0.27	4.09	1.5–27.3	66.29
Initial motility rating	3.27	0.03	0.54	2–5	16.51
Duration of "2" motility (*hr.*)	123.60	3.35	51.60	12–324	41.75
Total duration of motility (*hr.*)	197.13	5.40	83.18	36–612	42.20
Diluted semen					
Total duration motility (*hr.*)	445.89	8.60	132.34	180–876	29.68
Initial live sperm (%)	67.06	0.77	11.85	29–90	17.67
Live sperm following cold shock (%)	53.96	0.98	15.13	11–83	28.04
Fertility (81 samples) (%)	66.53	1.18	10.73	41–86	16.13

[a] Based on total variation.

sample and the percentage of live spermatozoa was determined after subjecting the diluted semen to a 0° C. temperature for 10 min.

Data from this study were analyzed by Fisher's analysis of variance, as described by Snedecor (9).

RESULTS

A characterization of the 236 semen samples studied is presented in table 1. The means with standard errors, standard deviations and ranges for each semen character studied are included in this characterization.

An analysis of variance of the data indicated that highly significant differences among bulls existed for all semen characters studied (table 2).

Since sufficient data were collected in this study on the individual bulls, it was decided to present the correlation analysis on a "total," "between" and "within bull" basis. This represents an extension of the usual "total" and

TABLE 2

Analysis of variance of data on semen characteristics

Variance source	Degrees of freedom	Mean square					
					Undiluted semen		
		Concentration	Volume	Total sperm	Initial motility	Duration of 2 motility	Duration motility
Total	235	226,793.5	2.42	16.73	0.29	2,662.46	6,919.73
Between bulls	18	1,433,430.1**	10.87**	75.12**	1.11**	5,799.44**	15,417.50**
Within bulls	217	120,098.18	1.72	11.89	0.22	2,402.24	6,214.84

	Degrees of freedom	Mean square			Degrees of freedom	Mean square
		Diluted semen				
		Duration motility	Initial live sperm	Live sperm, 0° C.		Fertility
			(%)	(%)		
Total	235	17,513.19	140.52	228.95	80	39.18
Between bulls	18	45,933.89**	409.94**	646.33**	13	296.85**
Within bulls	217	15,173.14	118.17	194.33	67	79.82

"grouped" data correlations presented in other studies of this type. Thus, data on all semen characters studied were correlated with data on initial percentage of live spermatozoa, percentage of live spermatozoa following the 0° C. cold shock and fertility (table 3). The coefficients of correlation show that, in most instances, the "between bull" correlations are higher than the "total" or the "within bull" correlations. Since there are fewer degrees of freedom involved in the "between bull" correlations, few of these achieve statistical sig-

TABLE 3

Tabulation of total, between and within bull coefficients of correlation among various semen characteristics and fertility[a]

Semen characteristics		Initial live sperm (%)	Live sperm surviving 0° C. (%)	Fertility
Undiluted semen				
Sperm concentration	Total	+0.07	−0.05	+0.23*
	Between bulls	−0.07	−0.25	+0.52
	Within bulls	+0.15*	+0.06	−0.07
Semen volume	Total	−0.02	+0.09	+0.20
	Between bulls	+0.02	−0.08	+0.37
	Within bulls	−0.04	+0.15*	+0.07
Sperm per ejaculate	Total	+0.08	−0.04	+0.22*
	Between bulls	−0.03	−0.21	+0.49
	Within bulls	+0.13	+0.03	−0.04
Initial motility	Total	+0.34**	+0.32**	+0.13
	Between bulls	+0.50*	+0.55*	+0.34
	Within bulls	+0.28**	+0.25**	+0.02
Duration of 2 motility	Total	−0.03	−0.01	−0.08
	Between bulls	+0.09	+0.26	−0.32
	Within bulls	−0.06	−0.07	+0.03
Total duration of motility	Total	−0.03	−0.04	−0.01
	Between bulls	+0.01	+0.18	−0.26
	Within bulls	−0.03	−0.09	+0.08
Diluted semen				
Total duration of motility	Total	+0.24**	−0.05	+0.25*
	Between bulls	+0.19	0.00	+0.25
	Within bulls	+0.26**	−0.07	+0.25*
Initial live sperm (%)	Total		+0.69**	+0.11
	Between bulls		+0.80**	+0.29
	Within bulls		+0.66**	0.00
Live sperm surviving 0° C. (%)	Total			+0.04
	Between bulls			+0.29
	Within bulls			−0.17

[a] All coefficients of correlation involving fertility are based on data from 81 semen samples from 14 bulls, while all intra-semen correlations are based on data from 236 semen samples from 19 bulls.

nificance. In addition to the coefficients of correlation listed in the table, additional correlations were determined between the percentage of spermatozoa killed by the 0° C. cold treatment and fertility where the percentage of spermatozoa killed by the cold shock is equal to the difference between the initial per cent live sperm and the per cent following the cold shock. These coefficients of correlation are as follows: "total," +0.06; "between bull," −0.13; "within bull," +0.27*.

For use in partial and multiple correlations, the following symbols were assigned to certain semen characteristics which had some degree of correlation with fertility: fertility, *1;* spermatozoa concentration, *2;* initial motility, *3;* duration of diluted motility, *4;* initial per cent live spermatozoa, *5;* and per cent live spermatozoa following 0° C. shock, *6*.

Multiple correlations involving fertility calculated on a "total" or "within bull" basis did not raise the zero order coefficients of correlation to any appreciable extent.

TABLE 4

Zero order and multiple between-bull coefficients of correlation involving fertility and selected semen characters

	Coefficients of correlation	Degrees of freedom	$r_{0.05}$ or $R_{0.05}$
r_{12}	+0.52	12	0.53
r_{13}	+0.34	12	0.53
r_{14}	+0.25	12	0.53
r_{15}	+0.29	12	0.53
r_{16}	+0.29	12	0.53
$R_{1.23456}$	+0.71	8	0.84
$R_{1.256}$	+0.70	10	0.73
$R_{1.25}$	+0.64	11	0.65
$R_{1.26}$	+0.69*	11	0.65

"Between bull" zero order and multiple coefficients of correlation of interest are presented in table 4. The multiple coefficients of correlation (R) involving fertility show that the percentage live spermatozoa following the 0° C. temperature shock and spermatozoa concentration contribute to the only statistically significant multiple correlation ($R_{1.26} = +0.69^*$). The further inclusion of semen characteristics *3, 4* and *5* does not materially increase this multiple correlation.

TABLE 5

Standard partial regression coefficients indicating relative influence of the various independent variables on fertility

	Standard partial regression coefficients		
All variables	Deleting independent variables 3 and 4	Deleting independent variables 3, 4 and 6	Deleting independent variables 3, 4 and 5
$b'_{12.3456} = +0.710$	$b'_{12.56} = +0.650$	$b'_{12.5} = +0.574$	$b'_{12.6} = +0.656$
$b'_{13.2456} = -0.120$			
$b'_{14.2356} = -0.009$			
$b'_{15.2346} = +0.154$	$b'_{15.26} = +0.126$	$b'_{15.2} = +0.372$	
$b'_{16.2345} = +0.435$	$b'_{16.25} = +0.391$		$b'_{16.2} = +0.476$
$R_{1.23456} = +0.71$	$R_{1.256} = +0.70$	$R_{1.25} = +0.64$	$R_{1.26} = +0.69^*$

Standard partial regression coefficients also were calculated to help assign relative importances of the independent variables. These are presented in table 5. A consideration of both the multiple correlations and the standard partial regression coefficients indicates that a partial regression equation for predicting the mean fertility levels of bulls ($\hat{Y}_1$) based on the independent vari-

ables, mean spermatozoa concentration (X_2) and the mean percentage of live spermatozoa following a 10-min. exposure to a 0° C. temperature (X_6), would be most meaningful. This equation is as follows: $\hat{Y}_1 = 32.40 + 0.0107\ X_2 + 0.3248\ X_6$.

Substitution of some mean values for the independent variables in the equation gives the following estimated mean fertility values:

X_2	X_6	$\hat{Y}_1$
1000	55	61.0
1000	65	64.2
1500	55	66.4
1500	65	69.6

These combinations of values indicate the possible usefulness of this equation for predicting mean fertility levels of bulls based on variations in mean spermatozoa concentrations and mean percentages of spermatozoa in diluted semen surviving a 0° C. cold shock.

DISCUSSION

The problem of selecting the semen samples to be used for artificial insemination continues to be a major one. Most studies to date have failed to provide the basis for predicting the fertilizing capacity of a semen sample before it is used in the field. The use of statistical treatment involving "total," "between" and "within bull" correlations, as well as partial and multiple correlations, has not been adequately investigated heretofore, and for that reason these analyses have been included in this study.

In the present study, the correlations of various semen characteristics with fertility obtained on the "total" and "within bull" basis are so low as to have little or no prediction value. This precludes the use of these correlations as a basis for predicting the fertilizing capacity of individual semen samples from bulls in general or from a given bull.

The usefulness of this study then is limited to predictions on a "between bull" basis. Thus, if from a given bull there are a series of semen samples on which determinations for spermatozoa concentration and the percentage of spermatozoa surviving a 0° C. cold shock have been made, the mean values for these two variables could be used in the partial regression equation to predict the relative mean fertility level for this bull. The correlation between actual and predicted mean fertility levels of a group of bulls similar to the ones used in this study should be approximately +0.69*, according to the multiple coefficient of correlation determined in this study. Thus, approximately 50 per cent of the "between bull" variance in regard to fertility is accounted for by this correlation involving spermatozoa concentration and the percentage of living spermatozoa following a 0° C. cold shock.

SUMMARY

The technique of differential staining of live and dead spermatozoa was utilized in this study to determine the relationship of the initial percentage of

live spermatozoa in semen samples, as well as the percentage of spermatozoa in diluted semen surviving a 0° C. temperature for 10 min., to other semen characters and fertility. Data on 236 semen samples from 19 bulls, including 81 samples from 14 bulls on which fertility data were based, were used in this study. An analysis of variance of the data for the various semen characters indicated that highly significant differences existed among the bulls in regard to these semen characters. A correlation analysis was presented on a "total," "between" and "within bull" basis, but only the analysis on a "between bull" basis had coefficients of correlation sufficiently high to have any prediction value. A multiple coefficient of correlation of + 0.69* of fertility with spermatozoa concentration and the percentage of spermatozoa surviving a 0° C. cold shock was obtained. A partial regression equation involving these factors was presented.

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DIFFERENCES IN MILK AND BUTTERFAT PRODUCTION AND TEST OF AYRSHIRE COW FAMILIES[1]

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The selection of dairy cattle for production may be based on the records of the individual, of the ancestors and other close relatives and of the progeny. The method where the records of the individual alone are considered is referred to as individual selection. On the other hand, the breeder practicing family selection considers the average of the family to which individuals belong and only individuals from the best families are saved, regardless of their individual merit.

Cow families which are considered to be outstanding in production or butterfat test have been discussed frequently in dairy magazines and breed journals. Bartlett and Margolin (1) noted that specific cow families and sires have the ability consistently to transmit genetic factors for superior size, butterfat percentage, milk production and total butterfat.

The object of this study was to compare a number of cow families to determine if there were significant differences between them in milk production, butterfat test and butterfat production. In addition, three methods of selection on the basis of cow families were compared with individual selection as a means of improving the average production of a herd.

The cow families in this study consisted of descendants of individuals which were the foundation animals at the time the herd was started. This is a common definition of cow families in use today, and is the one that is suggested most frequently for cow family analysis work.

EXPERIMENTAL PROCEDURE

The data consisted of records collected between 1922 and 1948 in the Reymann Memorial Herd of Ayrshires owned by the West Virginia Agricultural Experiment Station. The individual milk production, butterfat production and butterfat test records of 401 Ayrshire females were studied. These cows were progeny of 46 different sires and were members of 19 different cow families.

During the period covered, all milking cows were fed a home-mixed grain ration containing approximately 12 per cent digestible crude protein. The grain fed was limited to 14 lb. per day for cows and 10 lb. per day for first-calf heifers. The animals also were fed a good quality hay, silage when available and pasture in season. In addition, as far as possible, heifers were bred to freshen in the

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months of September, October and November, which helped to standardize feeding and management conditions.

The first available lactation record was used for each animal so that selection of records was not involved in this study. The lactation records were standardized to a 305-day, twice-a-day milking, mature-equivalent basis. In addition, the milk records were adjusted to a 4 per cent milk basis by the use of Gaines' (2) formula.

The cows in each of the 19 families were divided on the basis of their sire into subgroups. For example, all the cows in the *N* family that were sired by number 19 were in one subgroup. For the 19 cow families, the 401 cows in the production study made up 269 different sire-family subgroups. Both families and subgroups were analyzed by the analysis of variance (4) to test the significance of differences between families, between sires and between families within sires.

TABLE 1

The number of cows and their average milk (M.E. 4%) production, butterfat test and butterfat (M.E.) production for 19 cow families in the Reymann Memorial Herd from 1922–1948

Family	No. of cows	Milk M.E. 4%	Butterfat test	Butterfat M.E.
		(*lb.*)	(%)	(*lb.*)
A	31	9,748	4.30	402
B	17	9,596	4.34	383
C	29	9,388	4.31	387
D	22	9,144	4.27	375
E	14	9,127	4.16	371
F	35	8,873	4.30	364
G	45	8,828	4.35	365
H	18	8,786	4.16	356
I	12	8,729	4.33	360
J	24	8,634	4.11	350
K	25	8,498	4.18	346
L	13	8,454	4.26	346
M	19	8,454	4.17	343
N	10	8,381	4.44	350
O	13	8,068	4.32	334
P	37	7,988	4.24	326
Q	14	7,747	4.17	319
R	12	7,703	4.45	321
S	11	7,543	4.10	305

RESULTS

The milk (M.E., 4 per cent, 2×, 305 day) production, butterfat test and butterfat (M.E., 2×, 305 day) production averages for the 19 cow families in the Reymann Memorial Herd are given in table 1. Each family has ten or more cows in it and is represented by at least one member in the herd in 1947. There were other cow families which no longer exist. The milk production ranged from 7,543 lb. in the *S* family to 9,748 lb. in the *A* family. The butterfat test ranged from 4.10 in the *S* family to 4.45 in the *R* family, while the butterfat production ranged from 305 lb. in the *S* family to 402 lb. in the *A* family.

The daughter averages for the 46 sires of the 401 cows ranged from 5,903 to 14,417 lb. of milk, from 238 to 604 lb. of butterfat and from 3.90 to 4.73 per cent of butterfat.

The first production records of the 401 cows in the 19 cow families were analyzed by the analysis of variance and the results are shown in tables 2, 3 and

TABLE 2

Analysis of variance of M. E. 4% milk on 401 cows in the Reymann Memorial Herd

Source of variation	Degrees of freedom	Mean square	Per cent of total variance
Between families	18	7,638,888**	
Within families	382	3,647,736	
Between subgroups	268	4,655,839	
Between sires	45	14,791,738**	36
Between families within sires	223	2,610,479	8
Within subgroups	132	2,145,229	56
Total	400	3,827,338	100

** $P < 0.01$ (Highly significant).

4. In these tables the variation is divided into (a) variation due to differences between families, (b) variation due to differences within families, (c) variation due to differences between subgroups (cows in the same family and by the same sire) and (d) variation due to differences within subgroups, which is a measure of the average variation between records of cows in each of the subgroups. Variation due to *c* is further divided into differences between sires and differences between families within sires.

TABLE 3

Analysis of variance of butterfat test on 401 cows in the Reymann Memorial Herd

Source of variation	Degrees of freedom	Mean square	Per cent of total variance
Between families	18	0.169**	
Within families	382	0.091	
Between subgroups	268	0.106	
Between sires	45	0.220**	17
Between families within sires	223	0.083	8
Within subgroups	132	0.071	75
Total	400	0.094	100

** $P < 0.01$ (Highly significant).

The differences between families and also differences between sires were highly significant for milk production and butterfat test and were significant for the butterfat production. Part of the variation between families in milk production, butterfat test and butterfat production may be attributed to the fact that some sires were used more heavily on some families than on others.

Sire differences accounted for 36 per cent of the variation in milk and fat records and for 17 per cent of the variation in test. The differences between sires, however, are not caused entirely by variation in the breeding values of the sires, but also are influenced by environmental variations. The nature of the experiment does not permit a direct division between sire and year-to-year effect. Although every attempt was made to keep herd environment constant from year to year, it is highly probable that conditions that affect milk and fat production did change from year to year. In addition, it is possible that culling during the 26-yr. period aided in differentiating between families. However, this source of variation would be taken out in the between-sire portion of the variance. This may explain to some extent why sires appear to have been so important in distinguishing between families. The within-sire variation between families for milk production and test do give some indication, however, that some differentiation between families may have occurred. In tables 2 and 3,

TABLE 4

Analysis of variance of M. E. butterfat on 401 cows in the Reymann Memorial Herd

Source of variation	Degrees of freedom	Mean square	Per cent of total variance
Between families	18	12,891*	
Within families	382	7,310	
Between subgroups	268	8,860	
Between sires	45	29,228**	36
Between families within sires	223	4,749	
Within subgroups	132	4,926	64
Total	400	7,562	100

* $P < 0.05 > 0.01$ (Significant).
** $P < 0.01$ (Highly significant).

8 per cent of the total variation for both milk production and percentage of butterfat could be ascribed to differences between families within sires. However, the number of cattle in this study is too few to establish statistical significance for this source of variation.

Genetic relationship. In studying differences between families, it is important to know the genetic relationship of members of the same family. The higher the genetic relationship between members of the same family, the more the genetic variation will be between rather than within families. Also, the higher the genetic relationship, the more effective selection between families will be. The average genetic relationship (5) of members of the same family was computed to be 15 per cent. Since half-sisters, either paternal or maternal, and daughters and granddams are 25 per cent related, this relationship between members of the same family is not very high. In general, the genetic relationship decreases as the number of cows in the same family increases. However, the more heifer calves a particular cow has, the closer the family structure is woven, thus increasing the average genetic relationship.

Selection study. It was possible to make a comparison of a few theoretical selection methods because all normal females dropped in the Reymann Memorial Herd must complete their first lactation record before they can be culled. Therefore, at least one unselected record was available on all cows that freshened. These theoretical comparisons of different methods of selection were based on the first mature equivalent (M.E.) 4 per cent milk record of animals in the cow families. Individual selection was compared with three different methods of family selection. For these comparisons, selection arbitrarily was started in 1928 and comparisons were made for the two successive 10-yr. periods, 1928 to 1937 and 1938 to 1947. It was possible to obtain an idea of the outstanding families in 1928 by using the average production for each family between 1922 and 1927.

On the individual basis, two-thirds of the highest producing cows on the basis of their first lactation performance would have been selected as breeding stock each year, beginning in 1928, and the remaining one-third would have been culled. Once a cow was placed in either one or the other of the groups, she remained in this group until she left the herd, regardless of her future performance. If a cow was placed in the cull group, in practice, she would have been culled from the herd. Therefore, if she had any daughters in this herd after she was placed in the cull group, they automatically were placed in the group of culls and when they freshened, they were not considered in the division of the first calf heifers into the highest two-thirds and lowest one-third groups.

The three methods of family selection that were used are listed in table 5. The average milk records for the selected individuals and their descendants also are given in table 5. Individual selection would have increased the herd average from 7,632 lb. of milk (the 1922 to 1927 herd average) to 10,434 lb. of milk for the 1938 to 1947 herd average, or an increase of 2,802 lb. of milk. The offspring of the culls between 1938 and 1947 would have averaged 662 lb. of milk more than the 1922 to 1927 average of 7,632 lb.

Selection for milk production from the twelve highest-average producing families where only two-thirds of the highest cows in each family were kept with the lower one-third of the cows in each of these twelve families and the seven lowest-averaging families being culled, was the best method of family selection and would have increased the 1938 to 1947 herd average over the 1922 to 1927 herd average by 2,280 lb. of milk (9,912 minus 7,632). The offspring of the culls during the same period averaged 1,556 lb. of milk more than the 1922 to 1927 average.

If the twelve families with the highest average production had been selected and the seven families with the lowest average production had been culled, the 1938 to 1947 herd average would have been increased an average of 2,135 lb. of milk over the 1922 to 1927 average (9,767 minus 7,632). The offspring of the group of culls between 1938 and 1947 would have produced 1,333 lb. of milk more than the 1922 to 1927 average.

Where the third method of family selection was used, the 1938 to 1947 herd average was increased by 2,021 lb. of milk over the 1922 to 1927 average (9,653

minus 7,632). On the other hand, the progeny of the culls during the same period produced 1,768 lb. of milk more than the 1922 to 1927 average.

DISCUSSION

The differences between families for milk production, butterfat test and butterfat production all were significant. Part of this difference could be attributed to the fact that some sires were used more heavily on some families than they were on others. When the sire differences were eliminated, the remaining variations between families were not statistically significant. Thus, the sires contributed substantially to the differences between families—at least enough to

TABLE 5

Theoretical comparisons of selection methods based on the first M. E. 4 per cent milk record of animals in the Reymann Memorial Herd

Basis of selection	Average M. E. 4% milk production					
	1922–1927		1928–1937		1938–1947	
	No.	Av.	No.	Av.	No.	Av.
		(*lb.*)		(*lb.*)		(*lb.*)
Individual (on the yearly basis):						
⅔ highest producers	64	7,632	75	8,625	117	10,434
⅓ lowest producers (culls)			54	6,515	78	8,294
12 families with the highest average production between 1922 and 1927	44	8,121	105	7,704	149	9,767
7 families with the lowest average production between 1922 and 1927 (culls)	20	6,557	24	7,905	46	8,965
Highest ⅔ of the cows in each of 12 highest averaging families between 1922 and 1927	29	9,011	68	8,617	105	9,912
Lowest ⅓ of the cows in each of 12 highest averaging families, plus all members of 7 lowest averaging families between 1922 and 1927 (culls)	35	6,490	61	6,765	90	9,188
Highest ⅔ of the cows in each of 12 highest averaging families, plus highest ⅓ of the cows in each of 7 lowest producing families between 1922 and 1927	36	8,825	77	8,676	137	9,653
Lowest ⅓ of the cows in each of 12 highest averaging families, plus lowest ⅔ of the cows in each of 7 lowest producing families between 1922 and 1927 (culls)	28	6,098	52	6,358	58	9,400

make the differences significant. Although the differences between families within sires, which is a measure of the remaining differences between families after the sire differences are removed, are not significant, there are considerable differences left between families that may be important.

While the genetic relationships of members of the same cow family were rather low, this is somewhat offset by the fact that the environment was approximately the same for each family. Therefore, selection for differences between families in the same herd should be more effective than selection between families in different herds because differences between families in the same herd are

mostly genetic, if the environment is nearly random with respect to families (3). Thus, the environmental portion of records should be taken into consideration when differences between families kept in different herds are studied or compared.

The results of the selection study showed that individual selection for milk production was superior to family selection. Family selection where all members of a family were retained was not as effective in increasing milk production as when some individual selection was practiced within each family. Family selection would be expected to be superior to individual selection, if the phenotypic or observed correlation between members of the same family was very low and the genetic relationship of members of the same family was very high. The phenotypic correlation in these data for milk production was 0.05, for butterfat test, 0.04, and for butterfat production, 0.035. The average genetic relationship of these families was about 15 per cent. Lush (3) has developed a formula for comparing the relative effectiveness of family and individual selection. When the values for milk production were substituted into Lush's formula,[3] the results indicated that family selection could be expected to be 61.5 per cent as effective as individual selection in increasing the average milk production of the herd. The genetic relationship between members of the cow families in this herd would have to be about 28 per cent now for family selection to be equal to individual selection.

The genetic improvement in the producing ability of the Reymann Memorial Ayrshire cow families was due, in part, to the proved sires that were used on these cow families. The artificial insemination programs now make it possible for most dairymen to improve the producing abilities of their cattle by using the service of well-proved sires. Through this system of breeding, cow families will improve as well as the general level of production of herds in which the proved bulls are used.

SUMMARY

The milk production, butterfat test and butterfat production records of 401 animals that were members of 19 cow families were analyzed by the analysis of variance. The results showed that there were significant differences between the cow families in the three production figures studied. Most of these variations could be attributed to sire differences between families. When the sire differences were removed, the remaining variations between families were too small to be statistically significant. The differences between sires for the production figures were highly significant. Yearly variations contributed to the differences between sires. The average genetic relationship between members of the same cow family was 15 per cent.

Three methods of selection of breeding females on the basis of family su-

[3] $\frac{1+(n-1)\,r}{\sqrt{n\,[1+(n-1)\,t]}}$

n = number of individuals in each family.

r = genetic relationship between members of the same family.

t = phenotypic correlation between members of the same family.

periority in milk production were compared with selection on individual performance as a means of improving the production of the herd. Individual selection for milk production was superior to selecting females on a family basis. Family selections appeared to be more effective where some individual selection was practiced within the family than where all members of the best families were retained for breeding purposes.

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PROPERTIES OF THE COLOSTRUM OF THE DAIRY COW. V. YIELD, SPECIFIC GRAVITY AND CONCENTRATIONS OF TOTAL SOLIDS AND ITS VARIOUS COMPONENTS OF COLOSTRUM AND EARLY MILK[1]

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Considerable information on specific gravity and on concentrations of total solids, fat, protein, lactose and ash of colostrum has been accumulated. From the early studies, many of which are referred to by Houdinière (7), Overman and Sanmann (8) and Weber (18), there emerged a general picture of the gross composition of colostrum and a recognition of its variability. Within the past 25 yr., several studies of changes in the principal constituents of the mammary secretions during the transition period have been reported (3, 4, 5, 8, 12, 13, 15, 16, 17); however, none of these investigations included more than 15 animals. Van der Burg (19) of Holland published data on the composition of 163 colostrum samples collected over a period of 12 yr., but only the first postpartum milkings are included in his report. Hills (6) apparently was the first in this country to report results of colostrum studies.

In view of the limited amount of data on yields, specific gravity and concentrations of the major components in colostrum and early milk from breeds of dairy cattle common to this country, further study of the foregoing factors was considered desirable.

EXPERIMENTAL PROCEDURES

Feeding and management of cows. Samples of colostrum and early milk were obtained from 111 cows of four breeds, Holstein, Ayrshire, Jersey and Guernsey. These animals, all free of gross abnormalities, represented approximately two-thirds of the cows that calved in the College herd during the 3 yr. of the study. Cows that previously had lactated were given a conditioning period of 4 to 8 wk. before parturition.

The typical herd ration fed to most of the cows consisted of a concentrate mixture, Atlas sorgo silage and hay. Pasture grazing was allowed whenever it was available. Because of a period of grain scarcity, several cows received pasture as the principal feed prepartally, concentrates being fed only postpartally. Some cows received a winter barn ration that deviated from normal in that the levels of proteins in the concentrate mixture were either more or less than 16 per cent (9); others were supplemented with vitamin A and/or tocopherol concentrates (10, 11).

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Sampling procedure. In order that representative samples of the mammary secretions be obtained, calves were not allowed to nurse and the udder was evacuated as completely as possible by either hand or machine milking twice daily. The total collection at each milking during the first 14 days was weighed, and those selected for analyses were mixed and sampled carefully. If samples could not be analyzed immediately, they were stored at 4° C. for periods not to exceed 5 days. All samples were adjusted to a temperature of 20° C. and mixed before portions for analyses were removed.

Analyses made: (a) *Specific gravity.* This property was determined at 20° C. using a Mohr's balance. Though this procedure is considered to be less accurate than that employing a pycnometer, use of the latter is time-consuming. Values obtained in a series of tests of viscous colostrum using a pycnometer were either the same as or not more than 0.004 lower than those obtained by use of the balance.

TABLE 1

Results from different procedures of analyzing colostrum for fat content

Sample no.	Per cent fat		
	Babcock method		Mojonnier method
	Volumetric sampling	Gravimetric sampling	
C-1	1.65	1.60	1.79
C-2	2.90	2.80	2.86
C-3	3.85	3.70	3.88
C-4	2.90	2.80	2.81
C-5	7.35	7.25	7.28
C-6	.....	11.20	11.10
C-7	7.10	7.00	7.06

(b) *Total solids.* Approximately 3 ml. of the mammary product were weighed into a dish containing fine dry sand and heated at 100° C. overnight. The use of sand in the dishes was necessary to obtain reproducible results on replicate samples of viscous colostrum.

(c) *Fat.* Percentage of fat was determined by the Babcock method for whole milk. Comparisons of results on a series of samples using the Babcock and the Mojonnier procedures are shown in table 1. Although the methods yielded slightly different results on individual samples, the differences were so small that the more rapid and less laborious Babcock procedure was adopted.

(d) *Solids-not-fat.* This value was obtained by subtraction of percentage of fat from that of total solids.

(e) *Total protein.* Total protein values reported herein were obtained by subtraction of the sum of percentages of fat, lactose and ash from the percentage of total solids. For comparative purposes, protein in samples from twenty cows was determined by Rowland's (14) semi-micro Kjeldahl procedure and also by difference (table 2). By the latter method, average percentage protein was consistently higher than by the former, but only from 0.07 to 0.95 per cent. Thus,

calculations by difference seem to be adequate for studying the marked changes occurring in proteins of the secretions during the transition to normal milk.

(f) *Lactose.* The polarimetric A.O.A.C. method for lactose (1) was modified to the extent that the first 10 to 15 ml. of filtrate were discarded in order to obtain solutions suitable for polarization. In the case of samples of a high solids content, only a "one-*N* weight" of sample was used. Overman and Sanmann (8) also found the smaller samples sometimes advantageous.

(g) *Ash.* Ash was determined by evaporating 8 to 10 g. of sample to dryness in a porcelain dish and heating overnight at a temperature of 550° C.

RESULTS

Yields of colostrum and early milk. Average yields by groups of cows of four dairy breeds are shown in fig. 1. Except for a deviation at the second milking for Guernseys, the rates of change in production during the first 2 wk. were similar for all breeds. Quantities obtained at the first milking varied from 3.1 lb. (from a first-lactation Jersey) to 61.8 lb. (from a second-lactation Holstein). On a percentage basis, variations in yields within breeds were large in the first post-

TABLE 2

Comparisons of methods of calculation of concentrations of total protein in colostrum and early milk from 20 cows

Method of calculation	Per cent protein in milking no.								
	1	2	3	4	5	6	7+8[a]	15+16	27+28
Nitrogen × 6.38	16.46	10.30	5.88	4.59	4.16	4.12	3.96	3.48	3.19
By difference	17.41	10.93	6.38	4.68	4.32	4.28	4.12	3.61	3.26

[a] Sample composited on basis of yields.

partum milkings but decreased as total production increased. Average production during the first 2 wk. was highest by Holsteins and lowest by Jerseys, whereas that by Ayrshires and by Guernseys was similar but somewhat greater than that by Jerseys and less than that by Holsteins.

Specific gravity. Specific gravity of the early mammary secretions decreased rapidly in successive milkings, most of the change being completed before the fourth milking (table 3). Specific gravity of the secretions continued to decrease after the fourth milking, but at a less rapid rate.[3]

Lowest specific gravity values usually were found in secretions from Holsteins; however, marked variations were found within each breed. Four Jerseys (two of them first-lactation animals) and one Ayrshire produced first-milking colostrum having a specific gravity above 1.080. First-milking secretions from three other Jerseys, also first-lactation animals, had specific gravities between 1.027 and 1.034. In these latter secretions, concentrations of fat and of total solids were low. These properties, however, were normal by the end of the usual transi-

[3] Statements in this report concerning rates of change are based on graphic analysis (semi-log paper), which are not shown in order to conserve space.

TABLE 3

Specific gravity and concentrations of various constituents in colostrum and early milk

Breed	No. of milking							
	1	2	3	4	5+6[a]	7+8	15+16	27+28
				Specific gravity				
Holstein	1.056 (*29*)[b] ±0.01110[c]	1.040 (*29*) ±0.00536	1.035 (*29*) ±0.00287	1.033 (*29*) ±0.00193	1.033 (*29*) ±0.00130	1.033 (*27*) ±0.00119	1.032 (*25*) ±0.00114	1.032 (*5*) ±0.00187
Ayrshire	1.064 (*31*) ±0.01040	1.048 (*31*) ±0.00853	1.038 (*31*) ±0.00593	1.035 (*29*) ±0.00332	1.034 (*31*) ±0.00217	1.034 (*30*) ±0.00191	1.032 (*29*) ±0.00170	1.032 (*13*) ±0.00210
Jersey	1.063 (*32*) ±0.01439	1.049 (*32*) ±0.01131	1.040 (*32*) ±0.00580	1.036 (*32*) ±0.00283	1.035 (*31*) ±0.00152	1.035 (*29*) ±0.00133	1.034 (*26*) ±0.00147	1.033 (*6*) ±0.00141
Guernsey	1.065 (*19*) ±0.00776	1.045 (*19*) ±0.00683	1.037 (*19*) ±0.00383	1.035 (*19*) ±0.00325	1.035 (*19*) ±0.00141	1.034 (*19*) ±0.00187	1.033 (*18*) ±0.00129	1.031 (*4*) ±0.00173
				Solids (%)				
Holstein	23.9 (*10*) ±3.408	17.9 (*10*) ±2.042	14.1 (*10*) ±1.275	13.9 (*10*) ±0.923	13.6 (*10*) ±0.641	13.7 (*9*) ±0.975	13.6 (*10*) ±0.784	12.9 (*5*) ±0.687
Ayrshire	24.2 (*16*) ±3.803	21.8 (*16*) ±4.402	16.3 (*16*) ±2.332	15.1 (*15*) ±1.702	15.4 (*16*) ±2.049	15.3 (*15*) ±2.157	14.8 (*16*) ±1.692	14.0 (*12*) ±1.016
Jersey	22.5 (*16*) ±6.741	19.1 (*16*) ±5.769	15.3 (*16*) ±2.786	14.2 (*16*) ±1.374	14.4 (*16*) ±0.919	14.4 (*16*) ±0.971	14.3 (*14*) ±1.055	14.3 (*6*) ±0.574
Guernsey[d]	30.4 (*4*) ±1.229	22.8 (*4*) ±4.093	16.1 (*4*) ±1.133	14.9 (*4*) ±1.127	14.1 (*4*) ±1.253	14.7 (*4*) ±1.308	14.3 (*4*) ±1.207	14.4 (*4*) ±0.854
				Fat (%)				
Holstein	6.7 (*29*) ±2.650	5.4 (*29*) ±1.683	3.9 (*29*) ±1.038	4.4 (*29*) ±1.242	4.3 (*29*) ±0.724	4.4 (*27*) ±1.076	4.3 (*25*) ±0.787	4.0 (*5*) ±0.541
Ayrshire	5.1 (*31*) ±2.040	5.8 (*31*) ±2.306	5.0 (*31*) ±1.576	4.9 (*29*) ±1.290	4.9 (*31*) ±1.441	5.2 (*30*) ±1.736	5.2 (*29*) ±1.270	4.9 (*12*) ±0.891
Jersey	4.2 (*32*) ±1.812	5.0 (*32*) ±2.697	4.3 (*32*) ±1.539	4.3 (*32*) ±0.995	4.4 (*31*) ±0.858	4.7 (*29*) ±1.340	5.0 (*26*) ±1.153	5.0 (*6*) ±0.427
Guernsey	6.8 (*19*) ±1.780	6.1 (*19*) ±1.768	5.7 (*19*) ±1.233	4.7 (*19*) ±1.258	4.6 (*19*) ±1.296	5.0 (*19*) ±1.272	5.0 (*18*) ±0.821	5.2 (*4*) ±0.806

	No. of milking							
	1	2	3	4	5+6[a]	7+8	15+16	27+28
Solids-not-fat (%)								
Holstein	16.7 (*10*) ±3.029	12.2 (*10*) ±1.566	9.8 (*10*) ±2.812	9.4 (*10*) ±1.619	9.5 (*10*) ±0.402	9.3 (*9*) ±0.200	9.1 (*10*) ±0.521	8.8 (*5*) ±0.371
Ayrshire	19.1 (*16*) ±3.469	14.4 (*16*) ±2.399	11.1 (*16*) ±1.445	10.1 (*15*) ±1.202	9.9 (*16*) ±0.774	9.8 (*15*) ±0.442	9.4 (*16*) ±0.379	9.1 (*12*) ±0.464
Jersey	18.2 (*16*) ±5.574	15.0 (*16*) ±4.586	11.2 (*16*) ±2.258	10.3 (*16*) ±1.076	10.0 (*16*) ±0.352	10.0 (*16*) ±0.274	9.7 (*14*) ±0.325	9.4 (*6*) ±0.467
Guernsey[d]	23.0 (*4*) ±0.742	16.2 (*4*) ±2.918	11.1 (*4*) ±0.922	9.8 (*4*) ±0.153	9.7 (*4*) ±0.252	9.8 (*4*) ±0.224	9.4 (*4*) ±0.252	9.2 (*4*) ±0.163
Total protein (%)								
Holstein	14.0 (*8*) ±2.585	8.4 (*8*) ±3.133	5.1 (*8*) ±1.680	4.2 (*8*) ±0.748	4.1 (*8*) ±0,526	3.9 (*7*) ±0.328	3.4 (*8*) ±0.260	3.1 (*6*) ±0.325
Ayrshire	15.7 (*9*) ±1.542	9.9 (*9*) ±3.289	5.6 (*9*) ±1.459	4.6 (*9*) ±0.751	4.1 (*9*) ±0.423	4.2 (*8*) ±0.451	3.5 (*8*) ±0.360	3.1 (*6*) ±0.356
Jersey	14.2 (*11*) ±5.257	9.9 (*11*) ±4.510	5.8 (*11*) ±2.510	4.7 (*11*) ±1.080	4.4 (*11*) ±0.501	4.2 (*11*) ±0.333	3.9 (*10*) ±0.117	3.8 (*4*) ±0.784
Guernsey[d]	20.0 (*4*) ±0.666	11.9 (*4*) ±3.380	6.1 (*4*) ±1.058	4.4 (*4*) ±0.318	4.2 (*4*) ±0.065	4.2 (*4*) ±0.122	3.7 (*4*) ±0.238	3.3 (*4*) ±0.548
Lactose (%)								
Holstein	2.7 (*8*) ±0.911	3.9 (*8*) ±0.451	4.4 (*8*) ±0.208	4.6 (*8*) ±0.268	4.7 (*8*) ±0.212	4.8 (*7*) ±0.122	4.9 (*8*) ±0.100	5.0 (*6*) ±0.112
Ayrshire	2.6 (*9*) ±0.605	3.4 (*9*) ±0.737	4.2 (*9*) ±0.469	4.4 (*9*) ±0.357	4.7 (*9*) ±0.362	4.8 (*8*) ±0.224	4.9 (*9*) ±0.212	5.1 (*6*) ±0.037
Jersey	2.4 (*11*) ±0.771	3.3 (*11*) ±0.911	4.3 (*11*) ±0.697	4.7 (*11*) ±0.346	4.9 (*11*) ±0.226	5.0 (*11*) ±0.161	5.0 (*10*) ±0.182	4.9 (*4*) ±0.277
Guernsey[d]	1.8 (*4*) ±0.970	3.3 (*4*) ±0.535	4.2 (*4*) ±0.277	4.6 (*4*) ±0.342	4.7 (*4*) ±0.191	4.8 (*4*) ±0.208	4.9 (*4*) ±0.082	5.1 (*4*) ±0.100
Ash (%)								
Holstein	1.11 (*10*) ±0.158	.95 (*10*) ±0.081	.87 (*10*) ±0.045	.82 (*10*) ±0.047	.81 (*10*) ±0.026	.81 (*9*) ±0.034	.78 (*10*) ±0.067	.74 (*5*) ±0.036
Ayrshire	1.16 (*16*) ±0.147	1.07 (*16*) ±0.182	.95 (*16*) ±0.145	.91 (*15*) ±0.138	.89 (*16*) ±0.122	.89 (*15*) ±0.079	.82 (*16*) ±0.047	.77 (*12*) ±0.051
Jersey	1.22 (*16*) ±0.141	1.06 (*16*) ±0.149	.94 (*16*) ±0.082	.88 (*16*) ±0.052	.84 (*16*) ±0.036	.84 (*15*) ±0.034	.83 (*14*) ±0.039	.82 (*6*) ±0.036
Guernsey[d]	1.16 (*4*) ±0.217	.99 (*4*) ±0.119	.87 (*4*) ±0.035	.83 (*4*) ±0.029	.82 (*4*) ±0.031	.83 (*4*) ±0.029	.82 (*4*) ±0.026	.82 (*4*) ±0.024

[a] Sample composited on basis of yields; [b] No. of samples averaged; [c] standard deviation; [d] first lactation Guernseys only.

tion period. Colostrum having specific gravities of 1.025 and 1.028 was obtained from two second-lactation Holsteins. Part of the explanation for these low values was the fat content, 13 and 18 per cent, respectively. Several first-milking colostrum samples had specific gravities above 1.060 and fat content greater than 7 per cent, but frequently high fat values seemed to be associated with specific gravities lower than would be expected from concentrations of total solids.

Total solids. Solids decreased rapidly in the colostral secretions during the transition period (table 3). Rates of change were similar to those observed in specific gravity. Except in the first sample of colostrum, Holsteins produced secretions of the lowest content of solids. Content of solids in first-milking colostrum from the Guernseys was appreciably higher than that from the other breeds. Solids of first-milkings varied from 9 to 33.9 per cent.

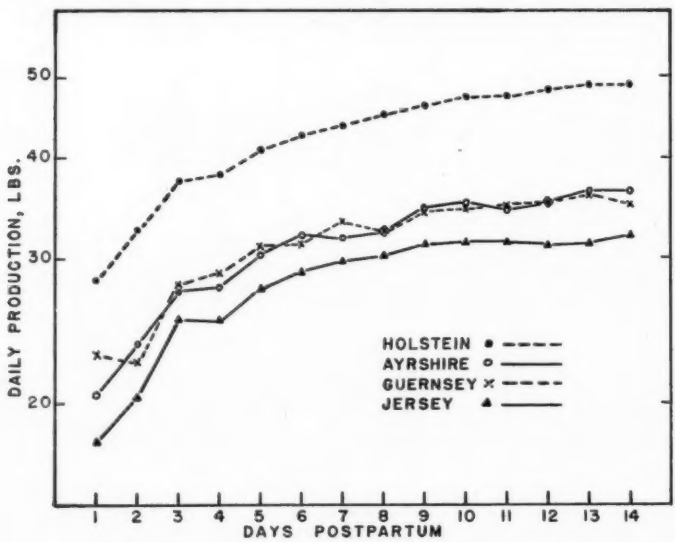

FIG. 1. Average daily production of colostrum and early milk by different breeds of cows. Samples were collected from 29 Holsteins, 31 Ayrshires, 32 Jerseys and 19 Guernseys.

Fat. Fat content of early mammary secretions was variable, not only among the different breeds (table 3) but also among individual cows of the same breed. The range in values of fat content of first-milking colostrum was from 0.3 per cent in a sample from a first-lactation Ayrshire to 12.5 to 18.0 per cent in samples from four Holsteins in first to third lactations.

Solids-not-fat. The principal difference found when data for solids-not-fat (table 3) and for total solids were plotted and compared was that smoother curves were obtained for the former. The variability of the fat content accounts for the difference. For the first three milkings, the solids-not-fat data from each breed closely followed a logarithmic decrease.

Total protein. Trends of changes in levels of proteins (table 3) were similar to those found for specific gravity and for total solids, the decreases following approximately a logarithmic path through the fourth milking. From this stage until the end of the period studied, further reduction occurred, but it was at a sharply reduced rate. Most of the decline in total proteins is traced to the reduction of the globulin content (9). The range of values for total proteins of first-milking colostrum was from 4.0 to 24.6 per cent; these extremes were obtained from first-lactation Jerseys.

Lactose. This constituent (table 3) changed inversely with specific gravity, solids, proteins and ash during the transition period. Lactose of first colostrum from Holsteins was highest and that from Guernseys was lowest, but differences in samples from the four breeds became smaller as the secretions changed from colostrum to normal milk. A sample of colostrum from a first-lactation Guernsey analyzed only 0.4 per cent lactose, which was the lowest value found; the highest was 4.1 per cent, in a sample from a first-lactation Holstein.

Ash. Changes in ash content of the mammary secretions (table 3) during the early part of the transition were not so marked as were those of specific gravity, solids and proteins. Extremes in ash concentrations of first colostrum were 0.81 and 1.47 per cent, in samples from a Holstein and a Jersey, respectively.

DISCUSSION

In all properties and constituents studied, early colostrum from different individuals was considerably more variable than was milk after the transition had occurred. Similar observations were made on proteins (9) and on vitamin A and carotenoids (10). Data in the literature, previously cited, also point to variation as a general characteristic of colostrum. General trends in composition of the secretions during the transition are in agreement with most of the earlier observations reported.

Fat was the most variable constituent studied. In consecutive samples from a number of animals, marked differences were found in concentrations of this component. Examination of production data along with those on fat content indicated that, in a number of cases, these variations might have been the result of incomplete milking. Use of oxytocin to stimulate let-down of milk (2) might have decreased the degree of variation in fat content noted.

In order to identify additional factors that caused variability in quantities and composition of the mammary secretions from different animals, the data (not shown) were arranged by breed, lactation number and season. During the early part of the transition, the average yields of mammary secretions were consistently lower for first-lactation cows than for those previously lactating. There was a tendency for production to be higher during the pasture-grazing season. In many cases solids were a little higher in secretions from first-lactation cows than from those previously lactating. Specific gravity, solids, fat and ash seemed to average slightly higher in samples collected during the winter barn-feeding period than in samples collected at other times. Other differences dependent upon number of previous lactations, season and/or type of ration were not indi-

cated by the data. Furthermore, variations in properties of secretions from animals of the same groups were so marked that the aforementioned differences should be regarded only as possible trends. Further studies are indicated before definite conclusions are justified.

In most cases, colostrum contained more nutrients than did milk, as a result of a higher concentration of proteins, minerals and sometimes fat. Colostrum from the Holsteins and the Guernseys had an energy content[4] about twice that of normal milk, a ratio similar to that calculated by Houdinière (7); but colostrum from Ayrshires and from Jerseys had an energy content only about 1.5 times that of the later milk. However, based on equal weights of dry matter, milk had an energy value either the same as or higher than first-milking colostrum. In agreement with an observation of Engel and Schlag (3), normal milk had a higher ratio of ash to total solids than did first-milking colostrum.

On an energy basis, proteins contributed two to three times more to the total nutritive value of colostrum than to that of normal milk. On the other hand, lactose in milk contributed 2.5 to 5 times as much to the total energy as it did in colostrum, whereas fat in milk constituted 10 to 50 per cent more of the total energy than it did in colostrum.

SUMMARY

A study was made of changes in yields, specific gravity, total solids, fat, solids-not-fat, total proteins, lactose and ash in mammary secretions collected from dairy cows during the transition period from colostrum to normal milk.

Considerable variation was found in values for yield and for the properties of early mammary secretions collected from different individuals at the same postpartal period. Fat was the most variable constituent. Variability in secretions from different individuals decreased as transition to normal milk progressed.

Yields of mammary secretions increased markedly during the first week postpartum.

Specific gravity, total solids, solids-not-fat, total proteins and ash decreased rapidly during the first four to six milkings, but only relatively small decreases were noted throughout the remainder of the 14 days of the study. Lactose changed in approximately an inverse ratio to the aforementioned properties.

Based on equal weights of dry matter, milk had an energy value the same as or higher than that of colostrum. Proteins contributed a greater percentage to the total energy value of colostrum than to that of milk, while lactose and fat contributed a greater percentage of energy to milk than to first colostrum. The ratio of ash to total solids was higher in milk than in colostrum.

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[4] Calculated from data of table 3 and the simple formula, relative energy value = protein + lactose + fat × 2.25.

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EFFECT OF INCUBATION TEMPERATURES ON THE RETENTION OF BACTERIOPHAGE BY A CULTURE OF *STREPTOCOCCUS LACTIS*[1]

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Mother cultures propagated in a dairy or cheese plant frequently fail to produce acid at a normal rate, as evidenced by the time required for the culture to coagulate milk. The duration of slow acid production by mother cultures is quite variable and may last for a few days or weeks, or the culture may never produce acid at its previous rate. When a mother culture fails to produce acid satisfactorily, it generally is discarded and a new culture obtained. However, the policy of some plants is to hold a slow acid-producing culture at a favorable growth temperature until coagulation occurs and then make further transfers from it. Future transfers from such a culture may show normal acid production after a few transfers. Cultures obtained from cheese plants and dairies experiencing difficulty with slow mother cultures for periods of rather short duration were found to contain bacteriophage. It appeared feasible to determine the duration of bacteriophage in a sensitive culture of *Streptococcus lactis* and to study some of the characteristics of the secondary-growth organisms resulting from the action of bacteriophage.

Nichols and Ineson (4) attributed starter recovery in commercial cultures to a mixture of several strains in the starter, some strains being unaffected by bacteriophage. Hunter (2) stated that the mechanism by which resistant forms originate after lysis of a culture by phage has not been explained satisfactorily. Yakovlev (5) noted that secondary-growth cultures were temporarily resistant to bacteriophage when grown on solid media; on liquid media their resistance was permanent but they remained carriers of bacteriophage. Hunter (2) found that under certain circumstances a phage-coccus mixture could be subcultured daily in milk and lysis did not occur. Such cocci, in the presence of symbiotic phage, showed resistance to lysis by other phage strains. Nelson *et al.* (3) state that the inhibitory principal (bacteriophage) may have a destabilizing effect on the cell when the changes involved in the loss of sensitivity characteristics are effected. The destabilized cell then may undergo either a gain or loss of sensitivity to various filtrates, the direction of the change being conditioned by factors at present unknown.

EXPERIMENTAL METHODS

Propagation of cultures. Cultures of *S. lactis* were transferred in skimmilk which had been autoclaved for 15 min. at 15 lb. pressure. An inoculation of approximately 1.0 per cent was employed.

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Determination of bacteriophage titer. Bacteriophage titers were determined on bacteria-free filtrates by the serial dilution method (1). The bacteriophage titer was expressed as the milliliters of filtrate required to cause a retardation in the production of acid, reduction of litmus or coagulation of milk by a sensitive culture of *S. lactis.*

Preparation of bacteria-free filtrates. Bacteria-free filtrates of cultures were prepared by filtering the coagulated milk cultures, under aseptic conditions, through coarse filter paper and then passing the filtrate thus obtained through a Selas micro-porous filter of #03 porosity.

TABLE 1

Coagulation times and bacteriophage titers of a culture of S. lactis, without and with added bacteriophage
(Incubated at 21° C.)

Transfer no.	Time required to obtain a firm coagulum (hr.)			Bacteriophage titer of culture receiving one inoculation with bacteriophage
	Control culture. Free of bacteriophage	Culture receiving one inoculation with bacteriophage	Culture receiving inoculation with bacteriophage at each transfer	
1	24	48	48	10^{-9}
2	18	23	23	10^{-8}
3	18	22	22	10^{-7}
4	18	18	19	10^{-4}
5	19	19	22	10^{-5}
15	22	24	22	10^{-5}
25	20	20	20	10^{-9}
35	21	27	27	10^{-5}
45	17	18	18	10^{-4}
55	19	19	20	10^{-8}
65	17	17	17	10^{-9}
75	17	17	17	10^{-1}
85	17	20	17	10^{-1}
95	15	15	15	10^{-1}
105	15	15	15	10^{-1}
115	16	16	16	10^{-1}
125	16	16	16	10^{-9}
132	16	16	16	< 1
133	15	15	15	< 1
134	15	15	15	< 1
135	15	15	15	< 1

Selection of test cultures. The pure cultures selected for study were rapid acid-producing strains isolated from commercial cultures. Only cultures showing rapid secondary growth were included in the study.

RESULTS

The coagulation times of a culture maintained in a bacteriophage-free condition, the coagulation times and bacteriophage titers of the same culture inoculated once with bacteriophage active against the organism and the coagulation times of the same culture given an inoculation with bacteriophage at each transfer are given in table 1.

The first transfer of the control culture (bacteriophage-free) coagulated milk 24 hr. sooner than the same culture inoculated with bacteriophage when an in-

cubation temperature of 21° C. was employed. The second transfer of the control culture coagulated milk 5 hr. sooner and the third transfer 4 hr. sooner than the cultures containing bacteriophage. Only slight variations in the coagulation times of all cultures were noted on the fourth and succeeding transfers. The culture inoculated once with bacteriophage had approximately the same coagulation time as the culture inoculated with bacteriophage at each transfer. The bacteria-free filtrate used in this experiment had a bacteriophage titer of 10^{-6} and the cultures were given a 1 per cent inoculation of the filtrate.

The bacteriophage titer of the culture receiving one inoculation with bacteriophage was 10^{-9} on the first transfer and showed a general decrease with succeeding propagations. However, the bacteriophage titer remained rather

TABLE 2

Coagulation times and bacteriophage titers of a culture of S. lactis, without and with added bacteriophage
(Incubated at 26° C. for 9 transfers and at 21° C. for the remaining transfers)

Transfer no.	Time required to obtain a firm coagulum (hr.)			Bacteriophage titer of culture receiving one inoculation with bacteriophage
	Control culture. Free of bacteriophage	Culture receiving one inoculation with bacteriophage	Culture receiving inoculation with bacteriophage at each transfer	
1	12	30	30	10^{-9}
2	14	20	20	10^{-9}
3	13	20	24	10^{-6}
4	14	18	18	10^{-8}
5	14	18	18	10^{-5}
6	14	17	20	10^{-6}
7	15	17	17	10^{-6}
8	14	12	12	10^{-6}
9	12	12	15	10^{-6}
10	12	14	14	10^{-6}
15	18	21	22	10^{-6}
20	21	22	21	10^{-4}
25	18	18	22	10^{-5}
30	18	18	18	10^{-5}
33	18	18	18	10^{-3}
34	20	20	20	10^{-1}
35	23	23	23	< 1
36	22	22	22	< 1
37	22	24	22	< 1

constant from the 75th to the 115th transfer; during this period the titer was low (10^{-1}). No bacteriophage could be detected in 1 ml. of a bacteria-free filtrate after 132 transfers.

The experimental data presented in table 2 were obtained with the same culture used in obtaining the data for table 1. Also, the experiment was carried out in the same manner except that the cultures were incubated at 26° C. for nine transfers and thereafter at 21° C. Table 2 shows that the first transfer of the control culture (bacteriophage-free) coagulated milk 18 hr. sooner than the same culture inoculated with bacteriophage. The difference in coagulation time between the control culture and the cultures containing bacteriophage became smaller with succeeding propagations. The culture receiving one inocula-

tion with bacteriophage, as well as the culture receiving an inoculation with bacteriophage at each transfer, coagulated milk in less time than the control culture on the eighth transfer. There were only small variations in the coagulation times of all cultures after the eighth transfer.

The bacteriophage titer of the culture receiving one inoculation with bacteriophage was 10^{-9} on the first and second transfers and then decreased. From the sixth to the 15th transfer the bacteriophage titer remained constant and then decreased. No bacteriophage could be detected in 1 ml. of bacteria-free filtrate after the 35th transfer. The bacteria-free filtrate used in this trial had a bacteriophage titer of 10^{-6} and the cultures were given a 1 per cent inoculation.

The data presented in table 3 give the coagulation times and bacteriophage titers of a culture of *S. lactis*, without and with added bacteriophage when incubated at 37° C. The culture employed in this trial was the one used to obtain the data presented in previous tables. Table 3 shows that the first transfer of

TABLE 3

Coagulation times and bacteriophage titers of a culture of S. lactis, without and with added bacteriophage
(Incubated at 37° C.)

Transfer no.	Time required to obtain a firm coagulum (hr.)			Bacteriophage titer of culture receiving one inoculation with bacteriophage
	Control culture. Free of bacteriophage	Culture receiving one inoculation with bacteriophage	Culture receiving inoculation with bacteriophage at each transfer	
1	5	76	76	10^{-7}
2	6	23	23	10^{-6}
3	7	24	26	10^{-6}
4	15	22	24	10^{-6}
5	15	15	15	10^{-6}
6	12	15	15	10^{-5}
7	24	24	32	10^{-1}
8	24	24	24	10^{-4}
9	12	19	20	10^{-3}
10	15	19	19	10^{-1}
11	15	19	19	<1
12	16	16	16	<1
13	16	15	15	<1
14	10	12	12	<1

the control culture (bacteriophage-free) coagulated milk 71 hr. sooner than the culture inoculated with bacteriophage. Marked differences in the coagulation times occurred on the second and third transfers also, but on the fifth transfer the coagulation times were the same for all cultures. Some variations in the coagulation times of the cultures were evident after five transfers but they were not great. The coagulation time of the control culture increased slightly for three transfers and an appreciable increase took place on the fourth propagation. The control culture had the greatest coagulation time on the seventh and eighth transfers and then the coagulation time decreased with some fluctuation.

The bacteriophage titer of the culture receiving one inoculation with bacteriophage was 10^{-7} on the first transfer and 10^{-6} on the second to fifth transfers.

The bacteriophage titer showed a general decrease with some variation until the 11th transfer. No bacteriophage was detected in 1 ml. of filtrate after the 11th transfer. The bacteria-free filtrate used to inoculate the cultures had a bacteriophage titer of 10^{-5} and the cultures were given a 1 per cent inoculation.

The bacteriophage titers of the cultures receiving an inoculation with bacteriophage at each transfer were determined but were not reported in the tables. In general, the bacteriophage titers of these cultures were relatively high for the first few transfers and then approached a value approximately equal to the titer of the bacteria-free filtrate added, taking into consideration the amount of dilution by the skimmilk employed as the growth medium. Also, the coagulation times and bacteriophage titers were determined on each transfer of a culture, even though all are not reported. The close correlation of results from day to day made it appear unnecessary to give data for each transfer.

The data presented in table 1 show that the bacteriophage titer of the culture receiving one inoculation with bacteriophage was 10^{-1} from the 75th to the 115th transfer and that the culture was not free of bacteriophage until the 132nd transfer. On the 113th transfer, two lots of the same milk were inoculated with the culture which received an initial inoculation with bacteriophage. One lot was incubated at 21° C. (data in table 1) and the other at 37° C. The culture incubated at 37° C. did not contain bacteriophage in 1 ml. of bacteria-free filtrate after one propagation, while the culture incubated at 21° C. showed bacteriophage present for an additional eighteen propagations. The results indicated that a culture carrying a small amount of bacteriophage for a rather long period of time at 21° C. quickly lost the bacteriophage when the incubation temperature was increased to 37° C.

The secondary-growth organisms resulting from the action of bacteriophage on a sensitive culture of *S. lactis* did not appear to undergo a change in morphology or colony characteristics. Sugar fermentations by the secondary-growth organisms were the same as for the parent culture. The secondary-growth organisms (free of bacteriophage) were not sensitive to the original bacteriophage which had caused lysis previously. The culture used to obtain the data presented in table 2 was used in a commercial cheese plant after it became free of bacteriophage with repeated transfer. The cheese plant selected was one which previously had difficulty with slow acid production due to bacteriophage. Although this culture was not sensitive to the original bacteriophage (phage #1) which had caused lysis, the culture developed sensitivity to another bacteriophage (phage #2) after it had been used in the cheese plant on 3 successive days. When phage #2 was added to another transfer of this culture that was carrying phage #1, no inhibition of the culture was evident, but when this culture became free of bacteriophage after repeated transfer, it became sensitive to the newly acquired strain of bacteriophage (phage #2).

DISCUSSION

The data reported in these experiments may help to clarify the instances of slow acid production, of rather short duration, frequently encountered in plants

where mother cultures are propagated daily for use in the manufacture of various dairy products. The results indicate that some cultures show a rather rapid secondary growth after attack by bacteriophage and behave quite normally after a few transfers. When a mother culture fails in a commercial plant which is carrying several cultures, it frequently is assumed that the culture was not inoculated by the person making the transfer. If only one culture is carried, it generally is thought that the culture became contaminated, that the incubator used may have failed to heat properly during the night and the temperature was too cold, etc. Many failures of mother cultures can be explained on the basis of bacteriophage.

In all of the experiments conducted, sterile skimmilk was employed as the growth medium. The sterile skimmilk showed various degrees of "browning" after sterilization and it was noted generally that the cultures required a slightly longer period of incubation to form a firm clot in the lots of skimmilk showing the most "browning." Therefore, slight differences in coagulation times are not significant. All cultures transferred on a particular day were from the same lot of milk and the comparisons between cultures receiving various treatments can be made with the control culture.

The appearance of secondary-growth organisms was more rapid at 26° C. than at 21 or 37° C. The results indicate that the rate of growth of the secondary-growth organisms is not related to the optimum growth temperature. The control culture, on the first propagation, coagulated milk in 24 hr. at 21° C., in 12 hr. at 26° C. and 5 hr. at 37° C.

The data presented in table 3 show that the control culture became progressively slower in acid production for the first eight transfers at 37° C. Later, it became somewhat more active. The culture used in these trials had been incubated at 21° C. previous to the time of these experiments. It has been noted that a culture carried at 21° C. does become somewhat slow when incubated at a higher temperature. Also, such a culture may not show its normal rate of acid production when returned to the lower temperature.

Studies on the secondary-growth organisms indicate that a culture which is carrying bacteriophage and producing acid quite normally may not be attacked by another bacteriophage strain until it becomes free of bacteriophage. The presence of bacteriophage in a culture may prevent a further attack by another strain of bacteriophage.

SUMMARY AND CONCLUSIONS

A study was made of the coagulation times and bacteriophage titers of a culture of *S. lactis*, without and with added bacteriophage, using incubation temperatures of 21, 26 and 21, and 37° C.

A culture of *S. lactis* inoculated once with bacteriophage active against the culture required 132 transfers before the culture became free of bacteriophage when incubation was carried out at 21° C. When the culture was inoculated once with bacteriophage and incubated at 26° C. for nine transfers and then at 21° C., it did not contain bacteriophage after 34 transfers. The same culture

inoculated once with bacteriophage and propagated at 37° C. did not contain bacteriophage after 10 transfers.

Cultures of *S. lactis* inoculated with bacteriophage active against the cultures produced acid rather rapidly after a few transfers, even though bacteriophage was present in the cultures.

Secondary-growth organisms resulting from the action of bacteriophage on a culture of *S. lactis* appeared to be similar to the parent culture in morphology, colony characteristics and in their ability to ferment sugars.

Secondary-growth organisms were resistant to the bacteriophage type causing incomplete lysis of the parent culture but were sensitive to at least one other bacteriophage type.

Secondary-growth organisms propagated in the presence of bacteriophage active against the parent culture were not sensitive to another bacteriophage type until the original bacteriophage had disappeared from the culture after repeated transfer.

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ABSTRACTS OF LITERATURE

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ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

399. Experimental studies on the incubation period of infectious abortion in cattle. A. THOMSEN, State Veterinary Serum Laboratory, Copenhagen, Denmark. Brit. Vet. J., **106,** 2: 41–54. Feb., 1950.

A study was made on the incubation period of bovine brucellosis and antibody formation by utilizing 24 mature heifers from brucellosis-free herds. Five heifers were infected at service by a bull in which infectious material (ground placenta in saline) was introduced in the prepuce just before breeding. The remaining animals were infected orally and by eye at various periods of gestation. The length of the incubation period was proportioned to the degree of development of the fetus at the time of infection. The average incubation period was 225 d. in heifers infected at breeding but only 53 d. in a heifer infected at 7 mo. gestation. The younger the fetus at the time of infection the longer is the period of incubation. The range of the incubation period varied from 44–251 d., depending upon the stage of gestation. A series of 23 graphs and 1 table are presented to show the data more effectively. B. B. Morgan

400. In vitro diffusion of penicillin from penicillin ointments for bovine mastitis. V. P. SEEBERG and J. P. STREET, Cutter Laboratories, Berkeley, Cal. Vet. Med., **45,** 4: 167–169. Apr., 1950.

In vitro diffusion studies were made on 14 different penicillin ointments used for bovine mastitis treatment. Optimum diffusion of penicillin occurred from those with bases composed of mixtures of white and liquid petrolatum. Concentration of the white petrolatum should be between 25–50%. Addition of beeswax greatly retarded the diffusion. B. B. Morgan

401. Mastitis from a veterinary practitioner's viewpoint. P. GAMBREL, Winnebago, Illinois. Vet. Med., **45,** 3: 122–124. Mar., 1950.

This is a review of the mastitis problem by a veterinary practitioner. The control of mastitis should be a joint responsibility of both dairyman and veterinarian. Some points are given in favor of the suspended-type milking machine over the claw-type milker for aid in the control of mastitis. It is suggested that the predisposing factor is trauma or injury to the upper part of the teat lining. This trauma is produced by improper use of a milking machine. All of the observations were based on 7 yr. experience in veterinary practice. B. B. Morgan

402. The recognition of ticke-borne fever as a disease of cattle. J. R. HUDSON, Ministry of Agriculture Veterinary Laboratory, Weybridge, England. Brit. Vet. J., **106,** 1: 3–17. Jan., 1950.

A mild, febrile disease of English cattle caused by a *Rickettsia* is described. The organism appears similar to *R. bovis* or *R. ovina.* Transmission studies were not made; however, the tick, *Ixodes ricinus,* has been incriminated. In cows inoculated with the organism, the incubation period ranged from 4–11 d. Although a mild disease which may upset milk production temporarily, it is not considered of much economic importance. Complete protocols are given.
B. B. Morgan

403. An outbreak of bovine leptospirosis in Pennsylvania. R. B. LITTLE, J. D. BECK and J. V. MCCAHON, Rockefeller Inst. for Med. Research, Princeton, N. J. Vet. Med., **45,** 3: 104–110. Mar., 1950.

A report is given on 7 outbreaks of bovine leptospirosis involving 33 cattle on different farms in Pennsylvania. Diagnoses were made by serological tests of the blood from infected cattle and the isolation of the organism (Leptospira) from guinea pigs injected with blood or milk from infected cows. The disease is characterized by

hemoglobinuria, anemia, icterus, fever abortion, loss of weight, decrease of milk production and moderate constipation. Morbidity varied from 10–50%, while the mortality rate was about 3%. Complete herd histories also were given. Recommendations for the control of this disease included (a) quarantine the herd and isolate each cow during the acute stage of the disease, (b) transfusion of 500 ml. of whole blood in sodium citrate from animals which have had the disease, (c) administration of penicillin and (d) daily administration of methenamine and sodium biphosphate. There is no evidence that the leptospira involved in these cattle is pathogenic to man; however, any outbreak in cattle should be regarded as dangerous until the organism is classified. B. B. Morgan

404. Infectious keratitis of cattle—a preliminary report. H. Farley, I. O. Kliewer, C. C. Pearson and L. E. Foote, Okla. Agr. Expt. Sta., Stillwater. Am. J. Vet. Research, **11,** 38: 17–21. Jan., 1950.

Hemophilus bovis has been isolated from numerous eyes afflicted with infectious keratitis and has been proposed as the cause of the disease. To test this hypothesis, 34 susceptible cattle were treated with pure cultures of *H. bovis* secured from infected eyes. None of the 34 developed keratitis. Of 29 calves treated with virulent secretions from keratitis cases, 8 developed the disease. *H. bovis* did not produce keratitis in rabbits or guinea pigs either. It is likely that *H. bovis* is not the primary etiologic agent in infectious keratitis, but only a secondary invader. E. W. Swanson

405. The transmission of anaplasmosis. G. Dikmans, U.S.D.A., Beltsville, Md. Am. J. Vet. Research, **11,** 38: 5–16. Jan., 1950.

A review of the various reported methods of transmitting anaplasmosis is presented. Transmission by ticks of widely differing species has been reported. However, very few of them support transovarian transmission, so the natural mode of transmission by ticks yet is obscure. The transfer of blood or infected material by needles, dehorning instruments, pitchforks, scalpels, etc. has been definitely responsible for numerous outbreaks. Transmission by flies, mosquitoes or other insects has been successful only when an infected animal or carrier has been in or near the susceptible herd so the insects can go directly from the infected to the susceptible animal. E. W. Swanson

406. Q fever studies in southern California. XI. Recovery of Coxiella burnetii from milk of sheep. W. L. Jellison, Rocky Mtn. Lab., Hamilton, Mont.; H. H. Welsh, Calif. State Dept. of Pub. Health; B. E. Elson, Calif. State Dept of Agr.; and R. J. Huebner, Natl. Institute of Health. Pub. Health Rept., **65,** 12: 395–399. Mar. 24, 1950.

A study of 300 cases of Q fever in southern California revealed 2 case histories with contacts suggesting sheep as the source of infection. The complement fixation test was run on 128 sheep in the flock to which 1 of these 2 patients had been exposed and only 33 were completely negative. The remainder gave low titer reactions, with none showing a positive reaction at a dilution greater than 1: 16. Q fever was produced in guinea pigs by injecting lacteal secretions obtained from these sheep. A strain of *Coxiella burnetii* was established in guinea pigs and identified by accepted criteria. D. D. Deane

407. Problems in cattle practice. W. J. Gibbons, Auburn, Ala. Vet. Med., **45,** 4: 147–150. Apr., 1950.

A brief review is given of some of the common ailments of cattle encountered over a 2-yr. period at the large animal surgical and ambulatory clinics at the Alabama Polytechnic Institute. Some of the disease conditions encountered were arsenic poisoning, anaplasmosis, acetonemia, bloat, malnutrition, mastitis, mycotic stomatitis, parasitisms, photosensitization, milk fever, sterility, screw worms and hyperkeratosis (X disease). Diseases discussed in detail were mycotic stomatitis, soremouth, milk fever, acetonemia and mastitis. B. B. Morgan

408. Omentectomy of cattle for studying insecticide residue in the body. R. D. Radeleff, Bureau of Animal Industry, U.S.D.A., Kerrville, Texas. Vet. Med., **45,** 3: 125–128. Mar., 1950.

An operation is described for removing portions of the omentum for chemical analyses. It has been shown that some of the newer insectisides are absorbed and stored in the fat of the omentum. This material may be collected and analyzed in the laboratory for the presence of chlorinated hydrocarbons. The choice of anesthesia is important. Chloral hydrate or chloroform cannot be used since their presence may obscure analyses. A 2% procaine hydrochloride is recommended. Of over 500 operations, only 2 terminated fatally. Repeated operations apparently do not interfere with the general health or weight gains of the animal. B. B. Morgan

BUTTER

O. F. HUNZIKER, SECTION EDITOR

409. Dairy engineering in the butter industry. A. M. PEARSON. Can. Dairy Ice Cream J., **29,** 3: 66–70. Mar., 1950.

The success of the operation of continuous butter making machines when processing neutralized cream is due to the "de-sludge" separator bowl. Formerly, 80% fat concentration was obtained only with sweet cream. A new stainless steel, No. 20, which is supposed to be more corrosion resistant than 18–8 has been introduced. Aluminum production has increased and cost has decreased so that more uses may be found for the metal in the dairy industry. Greater emphasis has been placed of late on the sanitary condition of equipment used in the manufacture of butter. Wooden butter equipment is being replaced by metal equipment. In the past few years the small, compact steam generator or packaged boiler has been used as the sole source of heat in the plant. In refrigeration the trend seems to be toward decentralization of refrigeration plants and towards multicylinder compressors operating at much increased RPM. Freon as a refrigerant seems to be gaining in popularity.

H. Pyenson

CHEESE

A. C. DAHLBERG, SECTION EDITOR

410. Cracked rinds in cheddar cheese. E. G. HOOD AND C. A. GIBSON. Can. Dairy Ice Cream J., **29,** 3: 29–30. Mar., 1950.

Cracked rinds in cheddar cheese may be caused by: (a) the development of excessively dry body; (b) high acidity; (c) low acidity due to inactive starter; (d) greasy curd; (e) temperature of curd at time of hooping under 70° F.; (f) use of previous day's curd; (g) hot water treatment in the hoop; (h) clogged press cloths and (i) failure of bandage and circle to meet.

H. Pyenson

411. Cheddar cheese storage plants. O. R. IRVINE. Can. Dairy Ice Cream J., **29,** 3: 90–94. Mar., 1950.

A major cost in assembling and storing cheese is that for labor. To reduce labor costs newer plants in Illinois and Wisconsin have gone to a 1-floor operation, except where processed cheese was being manufactured. Other labor costs can be saved by using fork trucks, storing cheese on pallets, high ceilings in storage rooms free from posts and blower coils for cooling. Advantage should be taken of automatic control equipment for refrigeration machinery. In the installation of insulating materials special attention is now being given to vapor barriers. H. Pyenson

412. Några iakttagelser vid paraffinering samt runmärkning av ost. (Some studies on the paraffining and Rune-branding of cheese.) G. LARSSON, Svenska Mejeriernas Riksförening Meddelande, **4.** Dec., 1947.

An automatic paraffining machine manufactured by the Wedholm factory, Model GE, was used in the experiments. The machine had 3 forks and each fork was designed to lower 1 cheese into the paraffin until it was well coated and then lift it to the table. Herrgårds, svecia and gouda cheese were used. In 1 hr. 200 cheese could be coated. The temperature of the paraffin was held at 150° C. for these experiments.

Some experiments in which the paraffining machine was not used were made to determine how long a time the cheese could be immersed in the paraffin without being damaged. The cheese could be in the hot paraffin for up to 10 sec. with no noticeable damage to it. A table was prepared to show the results from the experiments.

For the labeling of cheese with the "Rune" or Swedish national brand, an ordinary electric flat-iron was used. At the experimental dairy plant at Örnsköldsvik a test was made to determine the cost of labor. Three persons could label 100 cheese in 185 min., using 2 labels on each cheese. By using only 1 label and by arranging the work to save time and labor, the working time could be reduced about 35%. G. H. Wilster

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

413. Variations in the bactericidal and bacteriostatic properties of milk. A. D. MCEWEN AND MARJORY B. WHITE, Moredun Institute, Edinburgh. Vet. Record, **62,** 3: 27–30. Jan. 21, 1950.

This experiment was designed to determine if fluctuations in the bactericidal properties of milk occur which may account for degrees of resistance of the udder to infection. *Str. pyogenes* was the test organism used. Samples were taken from the R.H. and L.H. quarters of 1 cow for approximately a 2-mo. period during which time the R. H. quarter was injected with sterile saline, *Str. uberis* and *Str. dysgalactiae.* Mild inflammation caused by these injections did not alter the bactericidal and bacteriostatic titers of the milk. Studies on a different cow showed

the titers of the milk did not change with season or ration. Several cows showed a decrease in titers during the drying off period, but results did not indicate a difference between intermittent milking or complete cessation as a method of drying. R. P. Niedermeier

414. Preparing and maintaining good cultures. N. C. Angevine, Meyer-Blanke Co. Milk Plant Monthly, **39,** 3: 30–32. Mar., 1950.

Milk used in preparing cultures must be of excellent quality and contain 10–13% solids. Temperatures of at least 185° F. and holding periods of 30 min. to 1 hr. are necessary in obtaining proper pasteurization. Amount of inoculant will range from 0.25–2%, depending on the activity of the mother culture. Following incubation periods of 14–16 hr. at 70–72° F. good cultures will possess a smooth, firm texture, free of whey or gas, and an acidity of 0.80–0.85%. Cultures should also have a pleasant clean flavor that is mild but not pronounced.
J. A. Meiser, Jr.

415. Penicillin and other starter inhibitors. H. A. Ruehe, Univ. of Illinois, Urbana. Milk Plant Monthly, **39,** 3: 36, 38–39, 42. Mar., 1950.

Faulty cultures and delayed coagulation of cottage cheese may be caused by: (a) bacteriophage, (b) germicidal agents in cans and utensils and (c) antibiotics. Recently veterinarians have been injecting antibiotics such as penicillin into udders in the treatment of mastitis. To determine the effect of this drug on the quality of milk, as measured by the methylene blue reductase test, milks of high and low quality were inoculated with 4, 20, 80 and 160 units of penicillin/100 ml. of milk and methylene blue tests run on the samples. Presence of penicillin retarded decolorization of methylene blue but not sufficiently to change the classification of the milk. A suggested method for determining the presence of penicillin in milk involves pasteurization of a 10-ml. sample at 175° F. for 5 min., followed by cooling to 72° F. and inoculation with 1 ml. of starter. If no penicillin is present, a satisfactory coagulum should form in 10 hr. or less.
J. A. Meiser, Jr.

416. α-Acetolactic acid, an intermediate in acetylmethylcarbinol formation. (Abs.) E. Juni, Western Reserve Univ., Cleveland, O. Federation Proc., **9,** 1: 396. Mar., 1950.

Substantial evidence was obtained supporting the theory that acetolactic acid is the critical intermediate in the bacteriological conversion of pyruvic acid to acetylmethylcarbinol. A crude extract of *Aerobacter aerogenes* was resolved into 2 components, 1 capable of acting on pyruvic acid to form acetolactic acid and CO_2, the other decarboxylating acetolactic acid to acetylmethylcarbinol but without effect on pyruvic acid. Enzymatically produced acetolactic acid was shown to be identical with synthetic acetolactic acid. These findings were found to be consistent with the activity of preparations from other microorganisms. S. Patton

417. The nutrition of variants of Lactobacillus bifidus. R. M. Tomarelli, R. F. Norris and P. Gyorgy, Univ. of Pennsylvania, Philadelphia, and J. B. Hassinen and F. W. Bernhart, Wyeth, Inc., Mason, Mich. J. Biol. Chem., **181,** 2: 879–887. Dec., 1949.

L. bifidus, a predominant organism in the intestinal tract of infants fed human milk, grows better in culture media having milk added. Human milk was better than cow's milk. The growth factor in milk is associated with the unsaturated fatty acid fraction. Skimming did not decrease the activity. When both milks were digested with pancreatin, they showed an increase in growth activity and became equal in effect. A concentrate of the active constituents was obtained but not identified. At high levels, the digested cow's milk was inhibitory, but not so with the digested human milk. Both were found to contain steam-distillable inhibitory substances, but the digested human milk in addition had protective factors which neutralized the inhibitor. Growth factors for some of the strains of *L. bifidus* isolated are discussed. A. O. Call

418. Inhibition of growth of Lactobacillus casei by methionine and its relation to folic acid assimilation. G. Toennies, H. M. Winegard and D. L. Gallant, Lankenau Hospital Research Institute and The Institute for Cancer Research, Philadelphia. Arch. Biochem., **25,** 2: 246–256. Feb., 1950.

The role of methionine in the nutrition of *Lactobacillus casei* (ATCC 7469) has been studied with a view to clarification of contradictory findings reported by others. In the synthetic medium of Evans, as little as 10 γ of L-methionine/ml. medium effectively prolongs the lag phase of *L. casei* through its interference with the assimilation of folacin. During the early phases of growth the organisms were shown to hoard folacin; after accumulation has ceased, methionine was observed to accelerate growth by serving as a protein building block, sparing cystine. The inhibitory effect of methionine is considered to be a case of nutrient antagonism and is

unique in that it involves a vitamin and an amino acid, is not reversibly competitive, and the inhibitory concentration of amino acid is of a lower order of magnitude than that which is nutritionally effective. H. J. Peppler

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

419. A use of ascorbic acid in frozen homogenized milk. R. B. Anderson, C. W. Betzold and W. J. Carr, Sixth Army Area Food Laboratory, Seattle, Wash. Milk Plant Monthly, **39,** 3: 74–77. Mar., 1950.

To prevent the development of oxidized flavor concentrations of ascorbic acid ranging from 1.5 to 12.0 g. were added to 100 lb. of milk. Following pasteurization for 15.8 sec. at 164° F. and homogenization under 1,700 lb. pressure, the milk was cooled to 38° F. and stored in ice cream-style, deep freeze units at 0° F. for periods ranging from 30–90 d. Results indicated that 6 g. of added crystalline ascorbic acid/100 lb. of milk retarded the development of oxidized flavors and enabled the milk to retain appreciable amounts of ascorbic acid for 90-d. storage periods. Milk fortified to the 6-g. level that was properly handled could be shipped long distances and still be a good source of vitamin C.
J. A. Meiser, Jr.

420. Note sur le dosage des chlorures dans les laits. (A note on the determination of chlorides in milks.) M. Duron and A. Fournier. Lait, **30,** 291–292: 33–34. Jan.–Feb., 1950.

The procedure of Massot and Lestra for the determination of chlorides in milk is endorsed mainly because of sharpness in titration endpoint. Some minor modifications of the method, which appear to be advantageous, are presented.
S. Patton

421. Nouvelles recherches sur l'enzyme de Schardinger. (New studies of Schardinger's enzyme, xanthine or aldehyde oxidase.) L. M. Buruiana. Lait, **30,** 291–292: 2–24. Jan.–Feb., 1950.

A rather thorough review of known facts concerning the enzyme is presented at the outset. For the purposes of the study a method of separating the enzyme from milk was developed. The method appears to be convenient and to yield an enzyme extract of high strength and purity; essentially, it consists of extracting cream, which has been exhaustively washed by centrifuging and decantation of wash water, with a small quantity of 1% $NaHCO_3$ solution. Methods of preserving and properties of the enzyme extract prepared according to this procedure are given. Activity of the enzyme preparation is positively correlated with its P content. Enzyme activity was measured as time necessary to decolorize a standard methylene blue solution containing a known quantity of benzaldehyde.

The mechanism of the enzyme action in milk is elaborated by oxidation-reduction potential measurements using platinum and calomel electrodes. Donation of hydrogen by an aldehyde and acceptance of hydrogen by methylene blue or nitrates is demonstrated. Mathematical considerations are given relating the reduction phenomenon to an equation, $V = e^{-f/t/}$, where V equals the calomel electrode potential and ft is a function of time and variable temperature coefficients.
S. Patton

422. The quantities of amino acids in the nonprotein fraction of breast and cow's milk. R. J. Block and D. Bolling, N. Y. Medical College, New York. Arch. Biochem., **25,** 2: 350–353. Feb., 1950.

The approximate essential amino acid distribution in the nonprotein fractions of cow's milk (dry-skimmed and evaporated-skimmed milk) and human milk (3 mo. or longer post partum) was determined chemically and compared with the previously-reported amino acid patterns of the total proteins. Although breast milk contains about 4 times as much nonprotein nitrogen as does cow's milk, the essential amino acid patterns in the nonprotein fractions, as well as the total proteins, of the 2 milks show no significant differences. Calculations in terms of mg.% amino acids in milk or colostrum are presented to facilitate their use by nutritionists.
H. J. Peppler

423. Microbiological determination of isoleucine in proteins and foods. M. J. Horn, D. B. Jones and A. E. Blum, U.S.D.A., Washington. J. Biol. Chem., **180,** 2: 695–701. Sept., 1949.

A microbiological method for the determination of isoleucine is described. Results in good agreement with reported values were obtained using either *L. mesenteroides* or *S. faecalis* as the test organisms. Among the proteins and foods reported are values for casein, lactalbumin and dry skim milk.
A. O. Call

424. Standardizing the Babcock test for milk. E. O. Herreid. Can. Dairy Ice Cream J., **29,** 3: 78–80. Mar., 1950.

In order to standardize the Babcock procedure and make the test more accurate, the committee

appointed in the American Dairy Science Association has revised the test. By increasing to 18 ml. the volume of the sample of milk and eliminating the meniscus by the use of glymol, the Babcock test has been made in closer agreement with the ether extraction method. The results submitted by 5 collaborators on a total of 135 samples of unpreserved milk prove that the modified Babcock method is accurate.

H. Pyenson

425. Effects of anions, cations and amino acids on bovine alkaline phosphatases. (Abs.) C. A. ZITTLE and E. S. DELLA MONICA, Eastern Regional Research Lab., Phila., Pa. Federation Proc., **9,** 1: 251. Mar., 1950.

Studies of the activity and inhibition of alkaline phosphatases from cow's milk and from intestinal mucosa of the calf were made. Measurement of enzyme activity was based on liberation of phenol from phenylphosphate substrate. The results of the studies suggest that milk and intestinal phosphatases differ. S. Patton

426. Liberation of amino acids and active peptides from raw, heated and vitamin-free casein by tryptic digestion. (Abs.) R. A. SULLIVAN, W. E. DOWNEY, E. K. STANTON, E. VAN WAGONER and M. J. HANSELL, Natl. Dairy Research Labs., Oakdale, N. Y. Federation Proc., **9,** 1: 236. Mar., 1950.

Tryptic digestion revealed differences in the amount of amino acids liberated from a freshly prepared casein paste (46%), vitamin free casein (35%) and commercial casein (26%). Differences also were shown in non-dialyzable nitrogen and total weight of available amino acids in the respective hydrolysates. S. Patton

427. Studies on lactalbumin heated with carbohydrate. (Abs.) I. J. MADER, L. J. SCHROEDER and A. H. SMITH, Wayne Univ., Detroit, Mich. Federation Proc., **9,** 1: 365. Mar., 1950.

In vitro studies using pancreatin and *in vivo* studies with dogs indicated that heating lactalbumin with carbohydrate (part of the study concerning lactose) lowers the nutritive value of the protein. *In vitro* studies revealed that lysine was partially destroyed and that enzyme-resistant materials appeared to have been formed. Lowered digestibility was shown by increased fecal nitrogen in the *in vivo* studies. S. Patton

428. Liberation of amino acids and peptides from raw and heated bovine plasma albumin by pepsin and trypsin. W. H. RIESEN and C. A. ELVEHJEM, Univ. of Wisconsin, Madison. Arch. Biochem., **25,** 2: 335–346. Feb., 1950.

Amino acids and peptides released from raw and heated (to render it soluble during heating) crystalline bovine plasma albumin by crystalline pepsin and trypsin were assayed microbiologically with *Leuconostoc mesenteroides* P-60 and *L. citrovorum* 8081 and compared with the total release obtained by acid or alkaline hydrolysis. The peptic release of microbiologically-utilizable peptides was unaffected by previous heating of the albumin; however, heating increased the release of active peptides by trypsin. Maximum digestion was observed in the action of the pepsin-trypsin sequence on raw albumin or the trypsin-pepsin sequence on heated albumin. The extent of the release of peptides containing amino acids utilized by the assay bacteria varied considerably. Peptides containing utilizable tyrosine, valine and methionine were released most readily by pepsin, while arginine, tryptophane and methionine were rendered most available to the bacteria by trypsin. In this respect the results agree generally with those reported in studies with casein. Only negligible quantities of free amino acids were found in the peptic and tryptic digests of raw or heated albumin.

H. J. Peppler

Also see abs. no. 442.

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

429. Emulsionsproblemer ved maelk og flode. (Milk and cream emulsion problems.) A. B. WITTIG. Nordisk Mejeri-Tidsskrift, **15,** 7: 94–96. Aug. 15, 1949.

It is best to keep the pH of the milk product not lower than 6.6 for the production of the best emulsion by homogenization. Milk and cream should not have been subjected to storage for any length of time at temperatures of 10–50° C. before homogenization as this would cause damage to the natural emulsifier in the product. Homogenized products cannot be cooked for as long a period as unhomogenized products.

The color of milk changes from bluish-white to white during homogenization; this undoubtedly is due to the adsorption of casein during the increase of fat globule surface. The viscosity increases and the flavor becomes "fuller," richer and sweeter.

The illustrations show that the increased membrane surface area in milk as a result of the homogenization process causes an increased effectiveness in the product's emulsifying agents, a phenomenon not dissimilar to the one observed

when wetting agents are added to a fat-in-water emulsion. The article is concluded by the statement that the emulsion problems in connection with milk and cream do not cease at the dairy plant, but continue for a long time after the product has been marketed, and not least, after it has been consumed. G. H. Wilster

430. Milk filtration. H. INGLESENT. Food, **19,** 22: 25. Jan., 1950.

Experiments indicated that when modified starch was added to milk in quantities ranging from 0.1–0.5% and the milk recirculated in a plate and frame press until a filter coating developed, a useful filtration procedure was obtained. Bacteria numbers were significantly reduced; an "homogenizing effect" was noted, although fat content was not affected and extraneous matter was removed. K. G. Weckel

431. Practical piping problems. R. C. SORONEN. Heating, Piping, & Air Cond., **22,** 3: 104–106. Mar., 1950.

Installation of piping for processing requires planning not only in the routing of mains, but also for pipeline anchors, guides, supports and hangers. Several types of anchors, guides and supports are illustrated by drawings which show means of hanging, approximate dimensions and type of material used. A schedule for spacing of piping supports and hangers is presented.
H. L. Mitten, Jr.

432. Reviving sick compressors. H. C. WELCH. Operating Eng., **3,** 3: 36–37. Mar., 1950.

A brief step-by-step procedure for overhauling a vertical, single-acting, ammonia compressor is presented. The points covered are safety precautions, pumping-out of compressor, removal of cylinder heads, crankshaft bearings, cylinders and rings, pistons, valves, wrist pins and bushings, packing and assembly. H. L. Mitten, Jr.

433. License requirements for stationary and marine engineers. S. M. ELONKA, Operating Engineer, Albany, N. Y. Operating Eng., **3,** 2: 19–49. Feb., 1950.

The article is a tabulation of operating engineers' steam license requirements for states in the U. S., provinces in Canada, big cities of U. S. and Canada and the Merchant Marine. Headings of the requirements table are: States and cities, examination required, education and experience, class license, citizenship, local residence, non-local licenses recognized, fee and renewal and remarks. H. L. Mitten, Jr.

Also see abs. no. 409, 411.

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

434. Various forms of reduced delivery systems. G. E. FOOTE. Can. Dairy Ice Cream J., **29,** 3: 35–39, 102. Mar., 1950.

In Canada and the U. S. there are 6 major delivery systems being used not counting 7-d.-a-week delivery. They are as follows in order of their usage percentagewise: (a) every-other-day delivery (E.O.D.). Over 90% of all the home milk is delivered on this system in the U. S.; (b) 3-d.-a-week delivery; (c) 6-d.-a-week delivery (plant operation 7 d.); (d) 6-d.-a-week delivery (plant operation 6 d.); (e) 4-d.-a-week delivery; (f) 5-d.-a-week delivery. In the U. S. less than 3% of the milk devlivered to the homes is via the 7-d.-a-week system. In Canada it still is being used by 90% of milk delivery markets. The advantages and disadvantages of the various systems are given in detail.
H. Pyenson

435. Increasing plant efficiency through application of engineering practices. L. C. THOMSEN, Univ. of Wisconsin, Madison. Ice Cream Rev., **33,** 8: 48, 63. Mar., 1950.

The cost of producing a gallon of vanilla ice cream with 98% overrun, containing 10% fat, 10% serum solids, 16% sugar, 0.25% egg yolk solids and 0.2% stabilizer was estimated to be $1.146 in 1949. Of this amount, the ingredient cost amounted to $0.5164, labor, $0.3146, overhead, $0.2016 and operations, $0.1134.

The ingredient cost is based upon the approximate minimum compositional standards and maximum overrun consistent with the production of quality ice cream. Since this is no time for the industry to sacrifice quality, the possibility of reducing costs by reducing the fat content of the mix or by increasing overrun is out of the question. Any reduction in the price of mix ingredients is apt to be offset by increased labor costs.

Delivery costs have been reported to vary from a low of $0.2757/gal. for stops using 6000–7000 gal./yr. to over $0.90/gal. for the 100–200 gal./yr. stop. If delivery costs were to exceed $0.33/gal., a corresponding increase in selling price would appear necessary.

In 1947, the average annual wage paid to production workers in the ice cream industry was $2,347, as compared to $1,108 paid in 1939. The average wage cost for production workers per gallon of ice cream has increased from $0.057 in 1939 to $0.106 in 1947, despite the fact that the

output per worker during this same period increased from 19,578 gal. in 1939 to 22,138 gal. in 1947. Purchase of new equipment is justified whenever the labor which it saves will pay for the equipment within 3 yr.

Overhead costs includes depreciation, taxes, insurance, donations, travel, advertising and administrative expense. Since capital investment is responsible directly for depreciation and indirectly for other costs, it is important that a proper ratio (5:1) be maintained between yearly sales income and capital investment. Under present conditions, the capital investment should not exceed $0.30/gal. of annual output.

Possibilities of reducing operating costs are limited. A plant producing its own mix should manufacture a gallon of ice cream with not to exceed 0.3 kw. of electricity, 1.5 lb. of steam and 22 gal. of water. Use of evaporative condensers and S.T.H.T. pasteurizers might result in a saving of 4.5 gal. of water/gal. of ice cream produced. The installation of automatic boilers has proved advantageous in many plants. A good inventory system is essential in reducing waste and conserving supplies. W. J. Caulfield

436. Modalités et techniques de l'approvisionnement in lait des grands centres de consommation. (Modes and technics of supplying milk in large centers of consumption.) Lait, **30,** 291–292: 25–28. Jan.–Feb., 1950.

Certain problems relating to the distribution and consumption of pasteurized, evaporated and reconstituted dry milks in large population centers of France are discussed. S. Patton

Also see abs. no. 411, 412, 443.

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

437. Rumenal floral studies in the sheep. I. The nutritive value of rumen bacterial protein. F. M. Reed, R. J. Moir and E. J. Underwood, Univ. of Western Australia, Nedlands. Australian J. Sci. Research, Series B, **2,** 3: 304–317. 1949.

Rumen bacteria were separated from rumen fluid by differential centrifugation and then were dried in a hot air oven at 50–60° C. Proteins of dried rumen bacteria from sheep on "dry" feed, proteins of dried rumen bacteria from sheep on "green" feed and casein were compared in digestibility trials with rats. "True" digestibilities were found to be 64.8, 62.1 and 101.2 and biological values 77.9, 79.9 and 79.6, respectively, when fed at levels of 9.2, 9.7 and 9.5% crude protein. It is suggested that the value of microbial protein might be improved by adding sulfur-containing amino acids. G. E. Stoddard

GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

438. Storage of bull spermatozoa at low temperatures. Audrey V. Smith and C. Palge, Natl. Institute for Medical Research, Mill Hill, N. W. 7. Vet. Record, **62,** 9: 115–116. Mar. 4, 1950.

This study was designed to determine the effect of glycerol on the revival of bull and goat semen after freezing at very low temperatures. Motility alone was studied and no fertility trials were made. Glycerol was added to an egg yolk citrate diluent to give final concentrations of 5, 10, 15 and 20% glycerol. The diluted semen was cooled to –79° C. at different rates with a freezing mixture of solid CO_2 and alcohol. After varying periods the samples were thawed at +40° C. and compared with controls.

Ten per cent motility remained when glycerol-containing samples were frozen quickly at –79° C. and thawed after 5 min., while undiluted semen or diluted semen without glycerol showed no recovery. Revival increased when samples were cooled slowly, and were also greatest with higher glycerol concentration. Ninety per cent revivals were obtained with 15% glycerol cooled in 2.5-min. stages, with no difference noted between freezing time of 10 min. to 24 hr. Goat semen showed original motility following this treatment. R. P. Niedermeier

439. The fecundity of the immature rat following induced superovulation. C. R. Austin, Natl. Inst. Med. Research, Hampstead, London, England. J. Endocrinol., **6,** 3: 293–301. Jan., 1950.

Immature female rats, 30–45 d. old, each were injected subcutaneously with 20 international units (i. u.) of pregnant mare's serum, followed 56 hr. later by a subcutaneous injection of 20 i.u. of chorionic gonadotrophin. Following the second injection the females were placed with males. Dividing the rats into groups it was observed that 99% of the treated rats had ovulated. Only 34% of the treated rats had mated, however, and only 25% of the rats had fertilized eggs within 24 hr. after mating. The number of rats which showed implantation sites was 9%.

Although in these experiments superovulation was brought about with considerable success, the rate of fecundity was very low. Reasons for low fecundity were: (a) a failure to develop a complete state of estrus which led to a low incidence of mating and a poor transport of ova, (b) a low

rate of fertilization and (c) the shedding of abnormal eggs. V. Hurst

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

440. The seasonality of calf births in England and Wales. R. PHILLIPS, Univ. College of Wales, Aberystwyth. Brit. Vet. J., **106,** 1: 18–29. Jan., 1950.

A statistical study is presented of calf births recorded in the quarterly returns of the Ministry of Agriculture for England and Wales for the period from June, 1944, to June, 1948. A discussion is given on the monthly percentage of calf births in England and Wales, average percentage monthly calf births, average percentage monthly calf births per county, month of conceptions, relationship of alternate quarters and the peak calving months. The annual distribution of calf births was March and October.

B. B. Morgan

ICE CREAM

C. D. DAHLE, SECTION EDITOR

441. Isothermal and isobaric degassing of ice cream. A. LACHMANN, E. L. JACK and D. H. VOLMAN, Univ. of Cal., Davis. Ind. Eng. Chem., **42,** 2: 391–394. Feb., 1950.

The quantities of air liberated from ice cream of normal composition have been measured in 2 series of experiments: (a) by lowering the applied pressure isothermically at selected temperatures between – 10 and – 50 ° C. and (b) by raising the temperature isobarically at selected pressures between 760 and 300 mm. The maximum quantity of air liberated was related to both the temperature and pressure to which ice cream of a definite composition was subjected. A decrease in pressure or an increase in temperature brought the ice cream to a point where the air cells were fractured and the amount of liberated air was considerably increased. The rate of diffusion of air through the ice cream changed at the temperature and pressure differential point where cell fracture occurred. It was suggested that the results could be applied to a study of shrinkage in ice cream and that a quantitative determination of lamellae strength might serve as a guide to possible shrinkage. B. H. Webb

MILK AND CREAM

P. H. TRACY, SECTION EDITOR

442. Measurement of milk quality. A. G. LEGGATT. Can. Dairy Ice Cream J., **29,** 3: 86–88, 102. Mar., 1950.

A titration method for the estimation of the reducing capacity of milk has been developed. One ml. of a well-mixed sample is diluted with 10 ml. of distilled water and to this is added 1 drop of o-phenanthroline ferrous complex indicator (B.D.H. indicator). The sample is titrated to the discharge of the orange color by means of 0.01*N* ceric sulphate in *N* sulphuric acid solution from a micro burette. A pause of 30 sec., during which the orange color returns, is made and then more ceric sulphate solution is added until the same end-point is reached. The results of the titration may be expressed for convenience in milliliters of ceric sulphate. Greater values are obtained with abnormal milks.

H. Pyenson

443. Transportvägens (-tidens) inverkan på mjölkens hållbarhed. (Transportation (time) as it affects the keeping quality of milk.) T. BERGMAN and E. RAHMN. Svenska Mejeriernas Riksförening Meddelande, **6:** 1–8. Jan., 1948.

The centralization of the Swedish dairy manufacturing industry necessitates transporting milk over a greater distance. Three creameries (A, B and C) that received milk from long enough distances so that changes in the keeping quality of the milk during transit might be significant were chosen for study. The longest distance traveled was 8 Swedish (24 English) miles. Samples of milk were taken 3 times/mo., and about 98,000 samples were used for reductase tests during the investigations. An air-temperature record was made so that the effect of the temperature changes on the keeping quality of milk could be studied for every month of the year.

Long distance transportation tended to lower the quality of milk during the warm season of the year but not enough to be of concern to those who must depend upon long-distance transportation of their milk. G. H. Wilster

444. "Baseball contest" spurs milk sales. F. FLAGG. Milk Plant Monthly, **39,** 3: 52, 65. Mar., 1950.

To overcome the summer slump in sales, a baseball contest involving 10 six-man teams was created. With 2 teams playing against one another each week, the number of new quarts of business was the number of runs scored in each inning (weeks were divided into 7 innings). Weekly winners received $30, whereas the winner of the "World Series" at the end of 10 wk. received $200. J. A. Meiser, Jr.

445. Incentives boost milk sales. R. MILLER. Milk Plant Monthly, **39,** 3: 40–41. Mar., 1950.

To maintain routemen's enthusiasm at a high pitch and create sales when added business is especially desirable, routemen are paid $1 for each point gained over their base period. The highest-scoring routeman also receives 50¢ for each point scored by the other men in the contest which gives this plan a double-barreled effectiveness. J. A. Meiser, Jr.

Also see abs. no. 429, 430, 434, 436.

MILK SECRETION

V. R. SMITH, SECTION EDITOR

446. The effects of thyroxine and thio-uracil on the secretion of the phosphorus compounds normally present in milk. (Abs.) R. Chanda and E. C. Owen, Hannah Dairy Research Inst., Kirkhill, Ayr. Biochem. J., **44,** 4: xxix. 1949.

When cows were injected with thyroxine, the ester and lipid P content of the milk increased while phosphatase decreased. The reverse was true when thio-uracil was given. A table shows the average P distribution in the milk from control, thyroxine and thio-uracil groups before, during and following treatment. A. O. Call

NUTRITIVE VALUE OF DAIRY PRODUCTS

R. JENNESS, SECTION EDITOR

447. Factors influencing galactose utilization. V. H. Barki, P. Feigelson, R. A. Collins, E. B. Hart and C. A. Elvehjem, Univ. of Wisconsin, Madison. J. Biol. Chem., **181,** 2: 565–571. Dec., 1949.

Rats on whole milk diets excreted slightly less galactose than when on skim milk or skim milk plus cerelose diets. It is possible that the effect of the fat was to delay gastric emptying. The excretion of galactose was lower when an equivalent amount was fed in the form of lactose rather than as galactose. The major factor influencing the rate of galactose excretions seems to be the rate at which it reaches the circulation. A. O. Call

448. Significance of vitamin B_{12} in milk diets. (Abs.) R. A. Collins, L. S. Dietrich and C. A. Elvehjem, Univ. of Wisconsin, Madison. Federation Proc., **9,** 1: 355. Mar., 1950.

Inferior growth in rats fed a mineralized goat's milk diet compared to good growth promoted by a similar cow's milk diet was investigated. The differences in growth were explainable on the basis of superior contents of folic acid and vitamin B_{12} in cow's milk. Additions of vitamin C to the cow's milk diet increased B_{12} content of the livers, but showed no activity when added to the goat's milk diet. S. Patton

449. Recherches expérimentales sur la valeur antiscorbutique comparée des laits de vache frais, pasteurisés, bouillis, autoclavés, concentrés non sucrés, concentrés sucrés, secs non sucrés et secs sucrés. (Studies of the vitamin C content of raw, pasteurized, boiled, autoclaved, unsweetened and sweetened condensed, and unsweetened and sweetened dry milks.) L. Randoin and A. Perroteau. Lait, **30,** 291–292: 29–32, 33. Jan.–Feb., 1950.

Vitamin C determinations were made on samples of milk and milk products using a protein precipitant of trichloroacetic-metaphosphoric acids reagent and measurement of vitamin C in the filtrate by use of methylene blue. Results are given for several samples of raw and pasteurized milk treated in various ways. Untreated raw milk was observed to vary in vitamin C content between 15 and 28 mg./l. Pasteurized (in bottle) milk gave values slightly lower than those of raw milk; however, such treatments as boiling and autoclaving lowered appreciably the vitamin C content of both. The effects of heat on the vitamin C content of milk is discussed. Concentrated and dry milks, whether sweetened or unsweetened, contained only insignificant quantities of vitamin C. It is recommended that fruit juice be fed in conjunction with milk and milk products in order to supply satisfactory quantities of vitamin C in the diet. S. Patton

450. L'acide pantothénique dans les produits latiers. (Pantothenic acid in dairy products.) A. Houdiniere. Lait, **30,** 291–292: 37–40. Jan.–Feb., 1950.

A review with 27 references. S. Patton

451. Recherches expérimentales sur le lait de vache actinisé. III. Influence de l'actinisation sur la teneur du lait in vitamine A. (Investigations of irradiated milk. III. Influence of irradiation on the vitamin A content of milk.) J. Boisselot and J. Causeret. Lait, **30,** 291–292: 34–37. Jan.–Feb., 1950.

The object of the investigation was to study the effects of "actinisation," a rapid method of irradiation, on the vitamin A and carotene content of milk. Vitamin A was determined colorimetrically by the antimony trichloride method. Vitamin A and carotene were determined spectrophotometrically by absorption at 3280 Å. Total vitamin A activity was determined by bioassay. All three measurements indicated that

actinisation has little or no effect on the vitamin A content of milk. S. Patton

452. Milk is still our finest food. W. H. Riddell. Can. Dairy Ice Cream J., **29,** 3: 82–84. Mar., 1950.

This is a general discussion of the food value of milk. H. Pyenson

453. Amino acid content of evaporated milk on prolonged storage. A. Z. Hodson, Pet Milk Co., Greenville, Ill. Ind. Eng. Chem., **42,** 4: 694–695. April, 1950.

Samples of evaporated milk processed in the same plant but from different batches were analyzed for 17 amino acids. Fresh samples and samples that had been held in storage for 5 yr. were used. The amino acids were determined by microbiological methods. The only significant differences between the fresh and 5-yr.-old evaporated milks were losses of the aged samples in lysine (17%), histidine (17%) and arginine (11%). Analyses of 4 samples of evaporated milk held 15–17 mo. in storage indicated the losses of these 3 amino acids were not greater than 4% during this storage period. The losses in amino acids during the normal storage period for evaporated milk (less than 1 yr.) were considered nutritionally insignificant.

B. H. Webb

Also see abs. no. 427.

PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

454. Improved intravenous technique in the large animals. E. O. Longley. Vet. Record, **62,** 2: 15–16. Jan. 14, 1950.

Author describes a spear-pointed intravenous needle of hard stainless steel with a Record-type mounting used for both intravenous injections and bleeding. For intravenous injection an all-metal 2-way stop cock with male and female Record-type mountings is described and diagramed which makes possible complete initial assembly of apparatus prior to injection and simple adjustment of rate of flow using either positive pressure or gravity feed. Name of London firm manufacturing this needle is given.

R. P. Niedermeier

455. The sedimentation behavior of bovine and equine immune proteins. E. L. Smith and D. M. Brown, Univ. of Utah College of Medicine, Salt Lake City. J. Biol. Chem., **183,** 1: 241–249. Mar., 1950.

Sedimentation behavior of immune proteins, previously isolated by chemical methods, was studied in the Spinco electrically-driven ultracentrifuge. The principal component of these immune proteins from the cow and the horse had a sedimentation constant of about 7 Svedberg units (defined as 1×10^{-13} sq. cm./sec., and abbreviated as S). Associated with it a second component of about 10 S was observed. Similar results were obtained with bovine γ-globulin prepared by the electrophoresis-convection method. The authors reemphasize earlier statements that the same components occur in the various bovine and equine fractions whether obtained from milk, colostrum or plasma. H. J. Peppler

456. Partial specific volumes for some porcine and bovine plasma protein fractions. V. L. Koenig, Upsala Univ., Sweden. Arch. Biochem., **25,** 2: 241–245. Feb., 1950.

Bovine and porcine plasma protein fractions, prepared according to Cohn's procedure and previously examined ultracentrifugally, were employed in the determination of the apparent partial specific volume as indicated by the pycnometer technique of Drucker. The following average values of partial specific volumes were obtained for bovine fractions: fibrinogen, 0.706; γ-globulin, 0.725; β-globulin, 0.714; α-globulin, 0.722; crystalline albumin, 0.730. Average values for the porcine fractions were 0.744 (γ-globulin), 0.766 (β-globulin) and 0.731 for the impure albumin. The high values of porcine β-globulin may be due to traces of lipides. These values for bovine and porcine plasma protein fractions differ from those reported for human fractions.

H. J. Peppler

457. Studies on proteins from bovine colostrum. III. The homologous and heterologous transfer of ingested protein to the blood stream of the young animal. R. G. Hansen and P. H. Phillips, Univ. of Wisconsin, Madison. J. Biol. Chem., **179,** 2: 523–27. June, 1949.

Colostrums from the cow, goat and pig, taken immediately following parturition, were fed to new-born goats. Electrophoretic studies were made on the kids' blood before and after ingestion of each colostrum, and in each case there was an increase in "immune" proteins, but varying in degree for each source. These "immune" proteins seem to pass unchanged from the colostrum to the blood stream.

New-born calves were divided into 3 groups before nursing their dams. The control group

was fasted; a 2nd group was given mid-lactation milk; and the 3rd was fed cow colostrum. The serum of all calves at birth, showed the presence of proteins immunologically similar to "immune" proteins from colostrum; the amount, however, was increased by feeding milk and still further by feeding colostrum. A. O. Call

458. Molybdenum metabolism and interrelationships with copper and phosphorus. C. L. COMAR, L. SINGER and G. K. DAVIS, Florida Agr. Expt. Sta., Gainesville. J. Biol. Chem., **180,** 2: 913–922. Sept., 1949.

It was shown that in rats a high Mo intake retarded growth, but when accompanied by a high Cu intake growth was equal to groups on low Mo and low Cu; hence Mo toxicity must be considered in terms of the Cu level. Radioactive Mo^{99} and P^{32} were used to study their metabolism in the steer. Tables are given showing the tissue distribution of Mo and P when fed orally to 1 steer and when administered by jugular injection to another. A similarity between the behavior of these 2 elements in the bovine is pointed out. Radioisotopes of Mo, Cu and P were used in studying their interrelationships in the rat. Several probable mechanisms for Mo toxicity are postulated. A. O. Call

Also see abs, no. 425.

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

459. The sanitarian and the milking machine. T. R. ENRIGHT, Klenzade Products, Inc., Beloit, Wis. Milk Plant Monthly, **39,** 2: 68–70. Feb., 1950.

Prior to establishing an efficient, uniform procedure for cleaning and storing milking machines, accumulated deposits of milkstone must be removed by immersing all of the milker parts in a solution of 1 part organic acid cleaner and 4 parts warm water for 20 min., followed by vigorous brushing. A daily procedure that will maintain a physically clean machine is as follows: (a) flush equipment after milking with tepid water (100° F.); (b) dismantle machine and brush wash all parts in warm water (125° F.) to which an alkaline cleaner has been added; (c) every 4 d. substitute a balanced organic acid cleaner for the alkaline type in the brush washing procedure; (d) rinse the scrubbed parts in hot water (145° F.); (e) invert parts on metal drying racks and (f) prior to using, sanitize the assembled equipment with a liquid sodium hypochlorite solution. Wet storage solutions for rubber milking parts should be prepared using an organic acid detergent rather than lye or chlorine, since the latter compounds cause the rubber to become porous, thus preventing proper cleaning.

J. A. Meiser, Jr.

Also see abs. no. 408.

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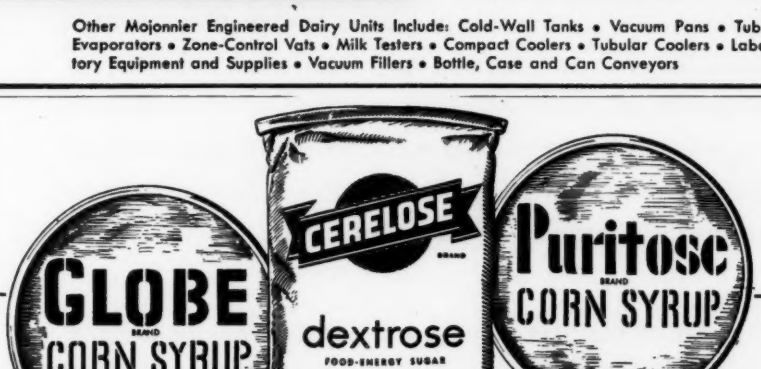

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GENERAL BIOCHEMICALS, INC.

20 LABORATORY PARK CHAGRIN FALLS, OHIO

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Culture Media for Examination of
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for Plate Counts

BACTO-TRYPTONE GLUCOSE EXTRACT AGAR is recommended for routine plate counts of bacteria in milk. This medium conforms to all requirements of "Standard Methods for the Examination of Dairy Products" of the American Public Health Association, except that it does not contain skim milk.

BACTO-PROTEOSE TRYPTONE AGAR is recommended for determinations of the total bacterial plate count of certified milk. This medium is prepared according to the specifications of "Methods and Standards for Certified Milk" of the American Association of Medical Milk Commissions.

for Detection of Coliform Bacteria

BACTO-VIOLET RED BILE AGAR is widely used for direct plate counts of coliform bacteria. Upon plates of this medium accurate counts of these organisms are readily obtained.

BACTO BRILLIANT GREEN BILE 2%

BACTO-FORMATE RICINOLEATE BROTH are very useful liquid media for detection of coliform bacteria in milk. Use of these media is approved in "Standard Methods."

for Detection of Molds

BACTO-POTATO DEXTROSE AGAR is an excellent medium for detection and enumeration of molds and yeasts in butter and other dairy products. The formula of this medium corresponds exactly with that specified in "Standard Methods."

BACTO-MALT AGAR is also widely used for determinations of the mold and yeast count of dairy products and for control of the sanitary conditions of manufacture.

for Cultivation of Lactobacilli

BACTO-TOMATO JUICE AGAR

BACTO-TRYPSIN DIGEST AGAR support luxuriant and characteristic growth of *Lactobacillus acidophilus*, and are well adapted for use in establishing the number of viable organisms in acidophilus products. These media are also widely used for estimation of the degree of implantation by *L. acidophilus*.

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